

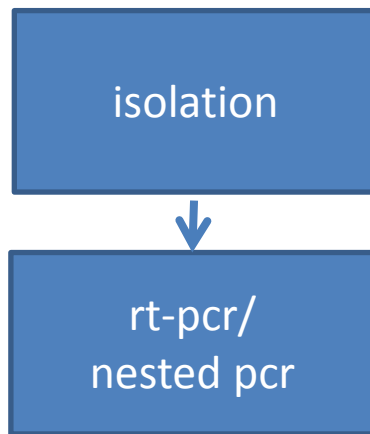


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One year of routine HIV-1 drug resistance testing by deep sequencing: insights from comparative Sanger sequencing.

introduction

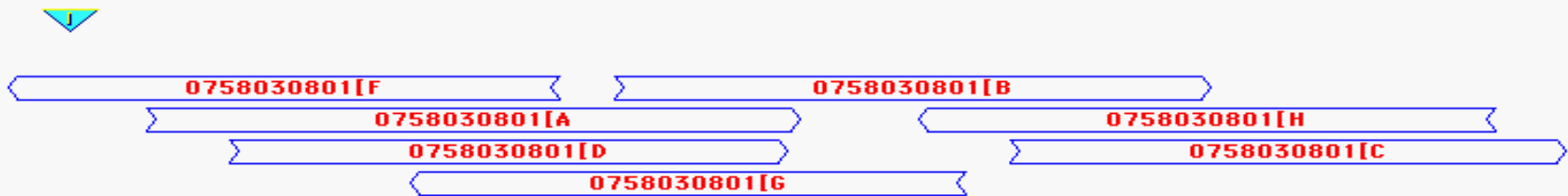
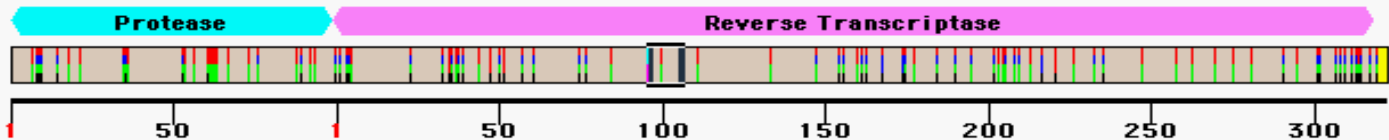
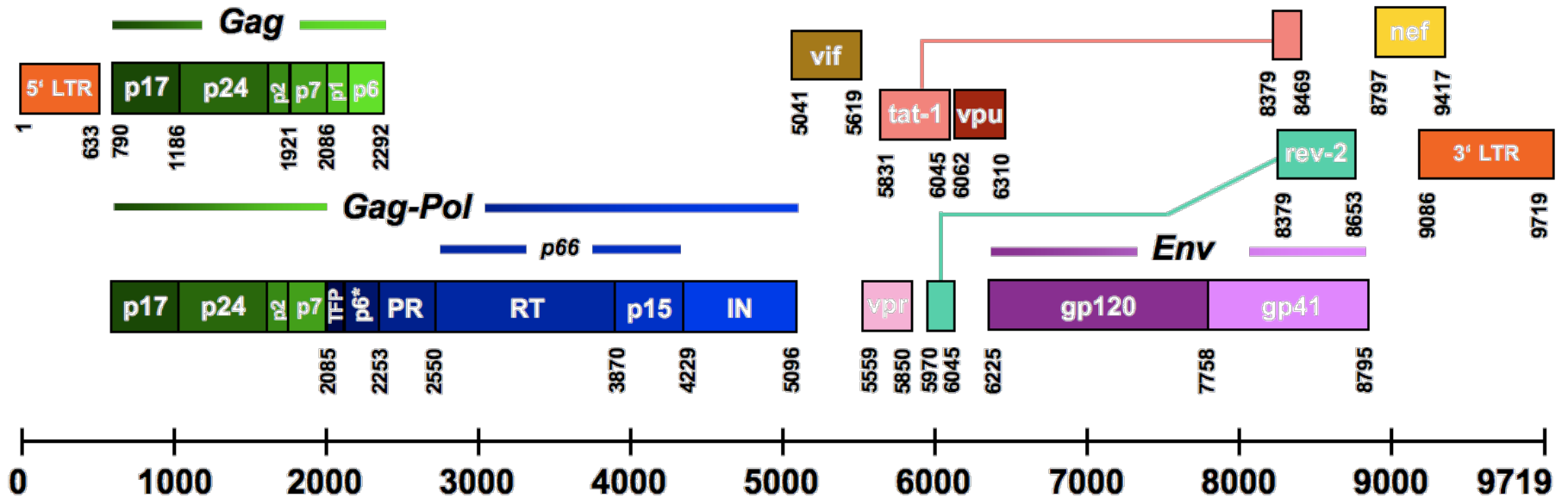
- drug resistance testing routinely performed prior to ART
- genotypic tests mainly based on population Sanger sequencing
 - limit of detection: 15-20%
- NGS technologies shown to be useful in studies
 - limit of detection: $\leq 1\%$ (possible)
- problems:
 - what are clinically relevant cutoffs? (is a 1% minority important?)
 - what is the real cutoff of Sanger sequencing? (which cutoff to apply for interpretation of NGS data analogous to Sanger?)

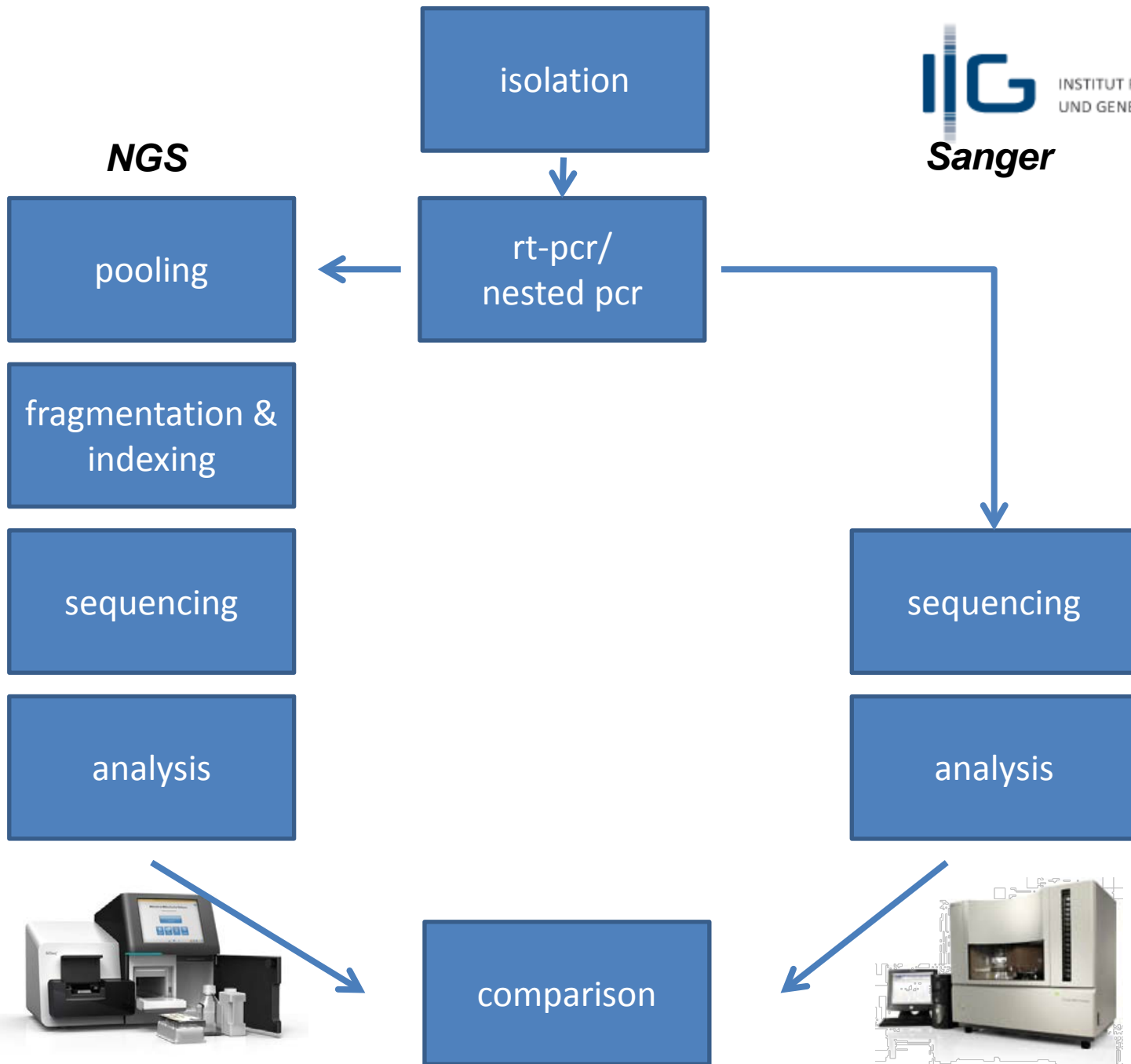


amplification



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validation

- NGS cutoffs used: 20%, 15%, 10%, 5%, 2%, 1%
- resistance mutations according to Stanford HIVdb (Dec 2013)
- tropism definition:
 - Sanger: geno2pheno[coreceptor] using cutoffs:
 - German treatment guidelines
 - NGS: local geno2pheno[454] implementation using cutoffs:
 - $\geq 2\%$ X4 at 3.5% FPR according to Swenson et al., 2011
- resistance interpretation using local HIV-GRADE implementation

results

- >400 samples analyzed with Sanger & NGS:
 - 312 PR/RT
 - 27 INT
 - 97 V3
- median coverages:

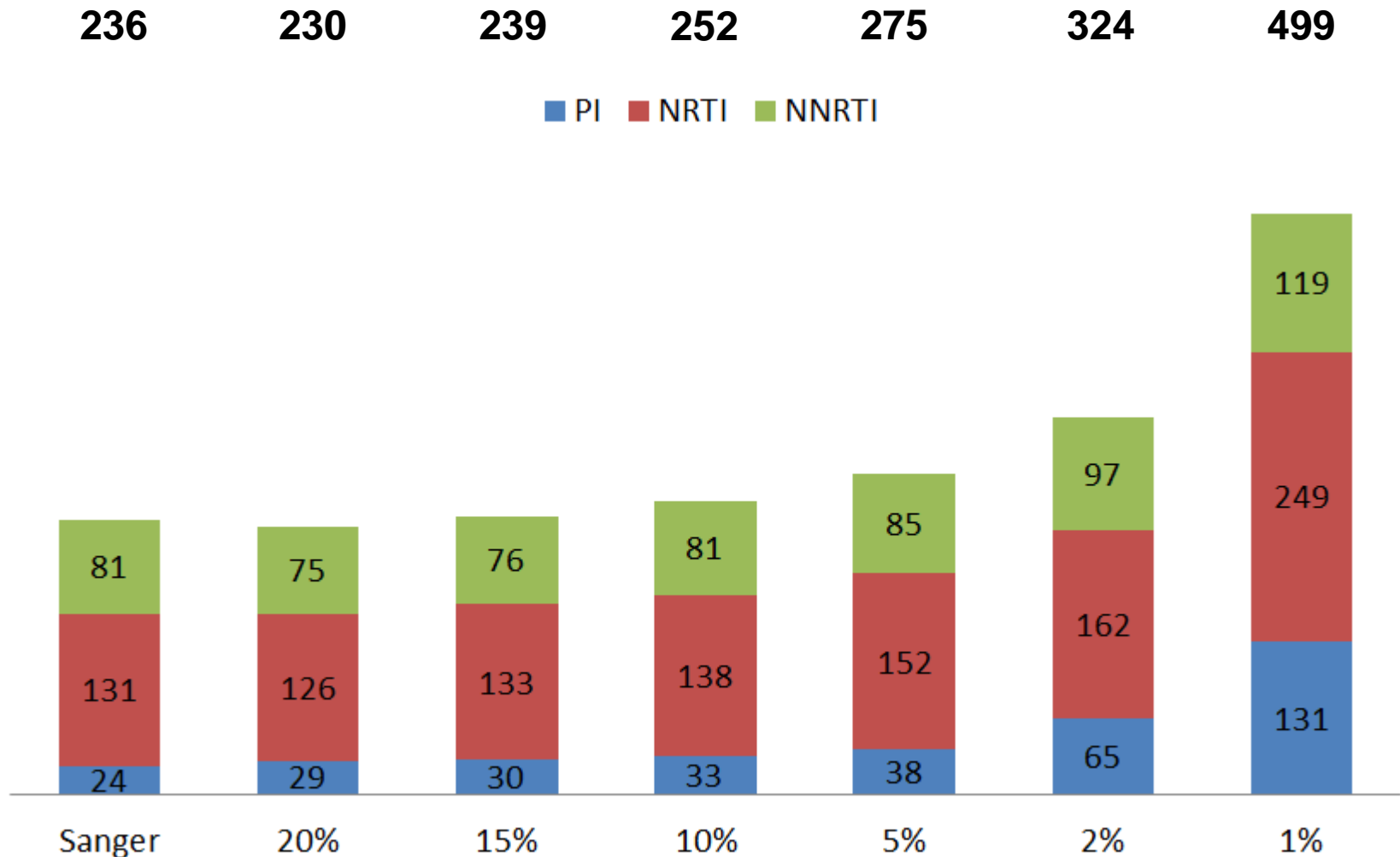
| | |
|------------|-------|
| • PR / RT: | 29655 |
| • INT: | 15437 |
| • V3: | 39571 |

protease & reverse transcriptase

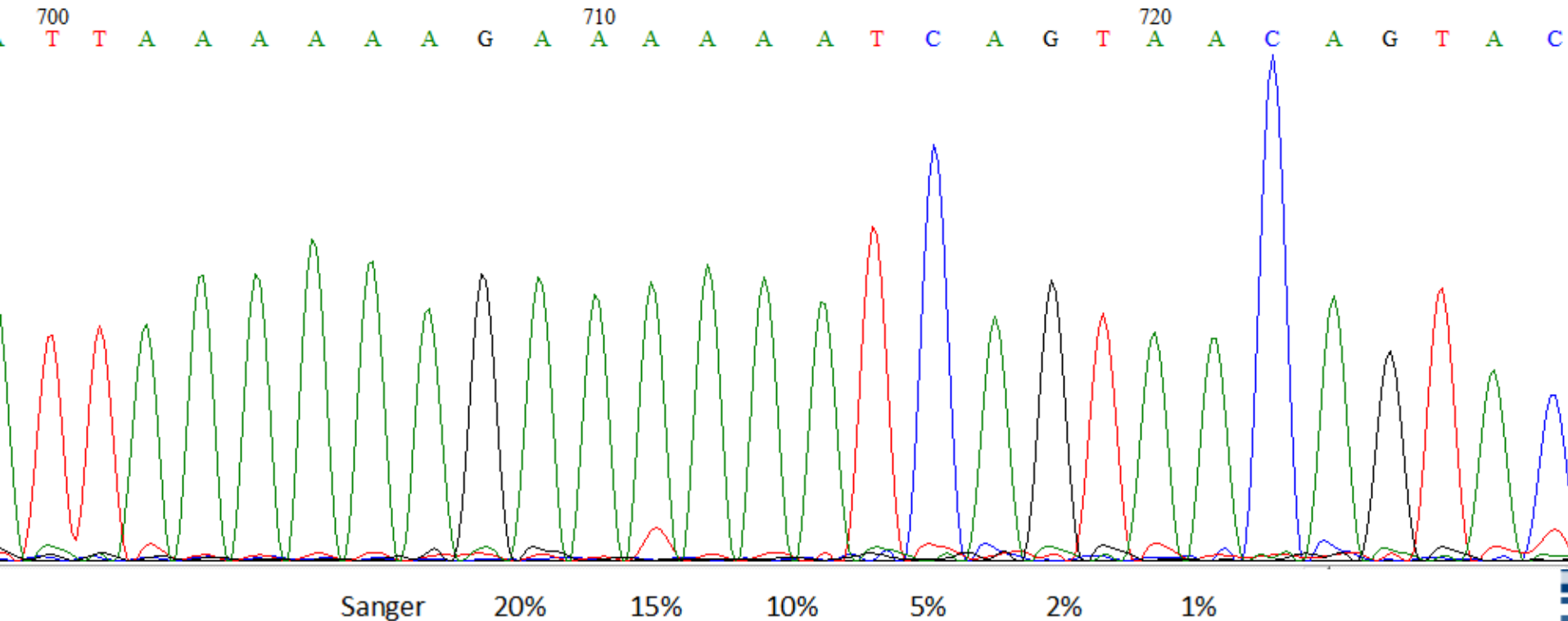
PR/RT – # resistance mutations found



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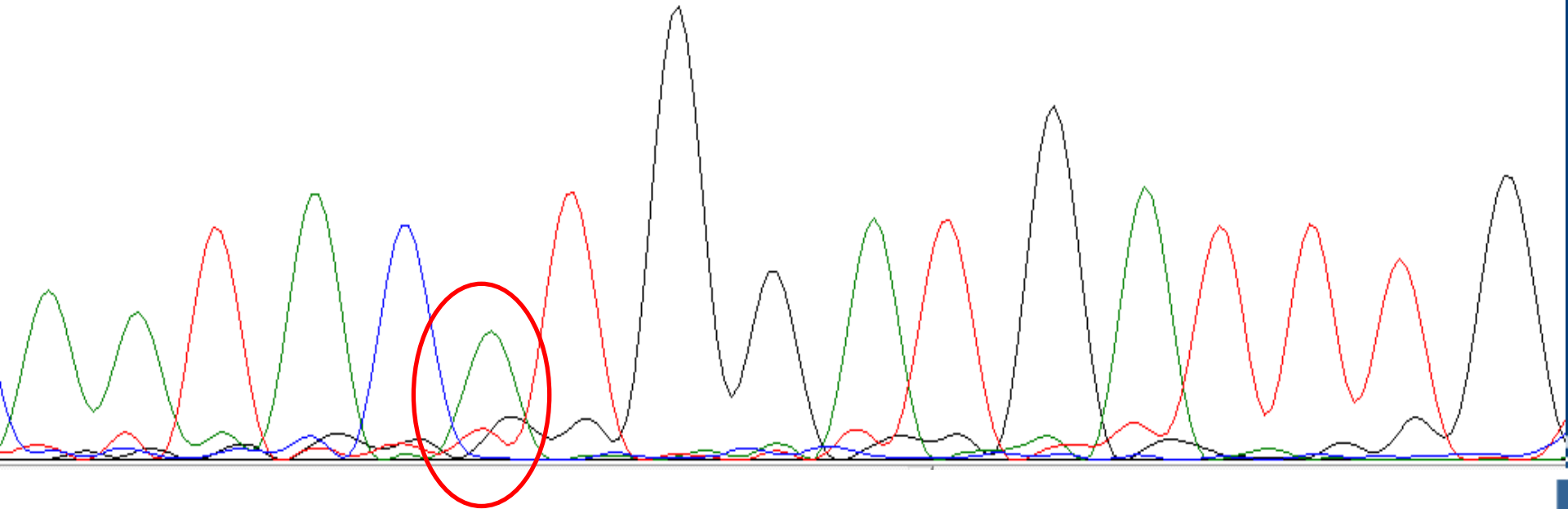


PR/RT – resistance mutations



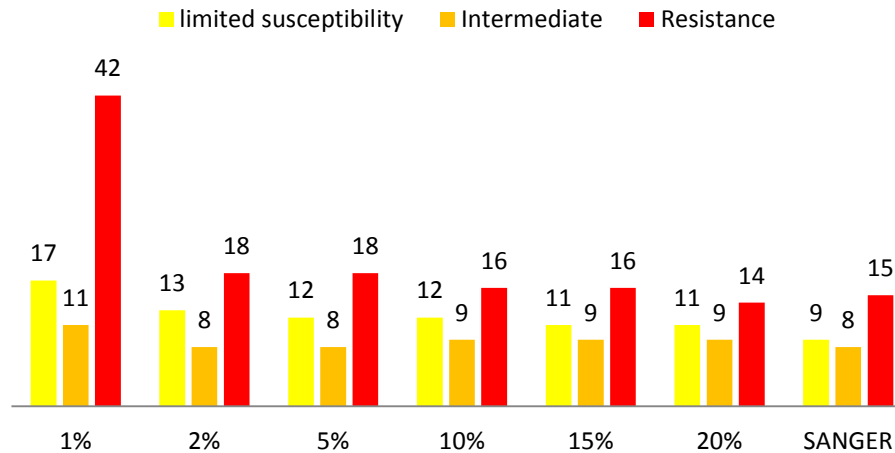
PR/RT – resistance mutations

A A T A C A T G G A T G A T T T G

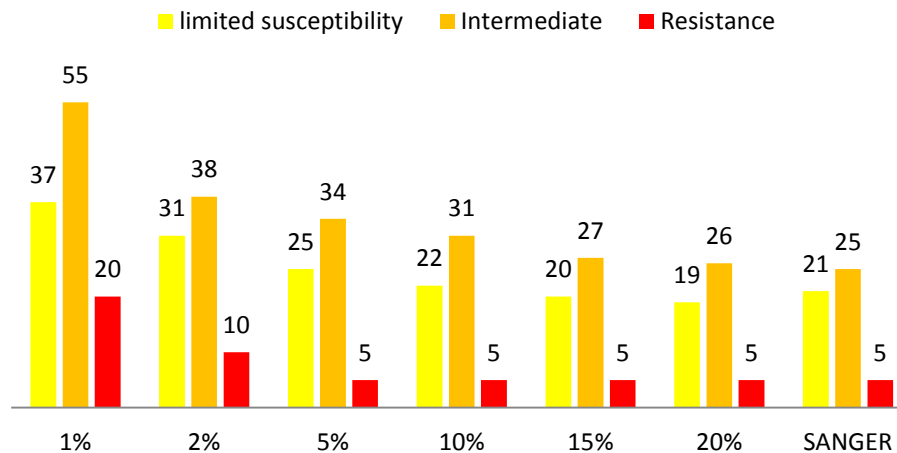


PR/RT – resistance interpretation

TDF



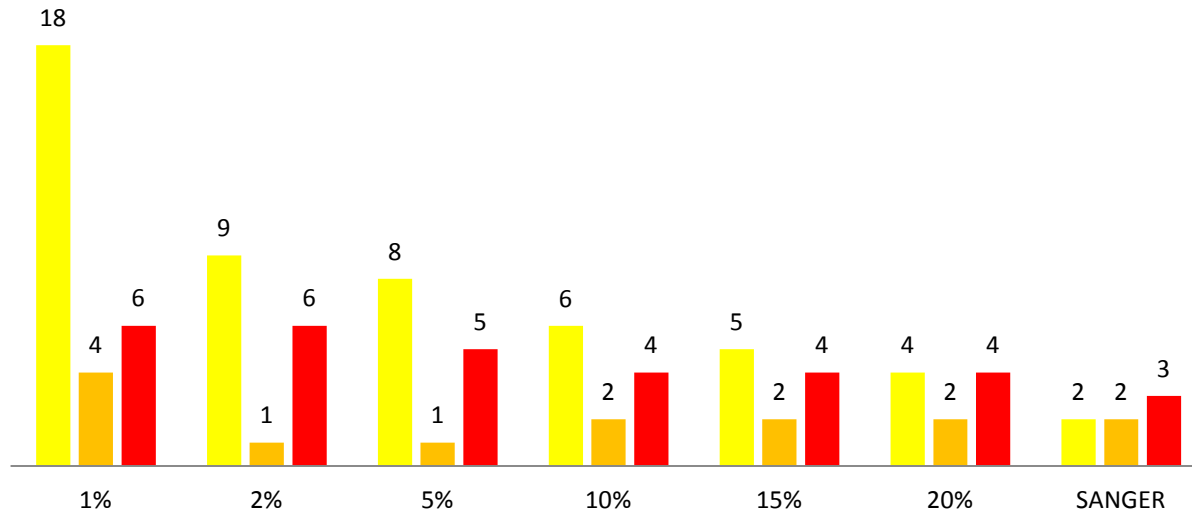
RPV



PR/RT – resistance interpretation

ATV/r

■ limited susceptibility ■ Intermediate ■ Resistance



integrase

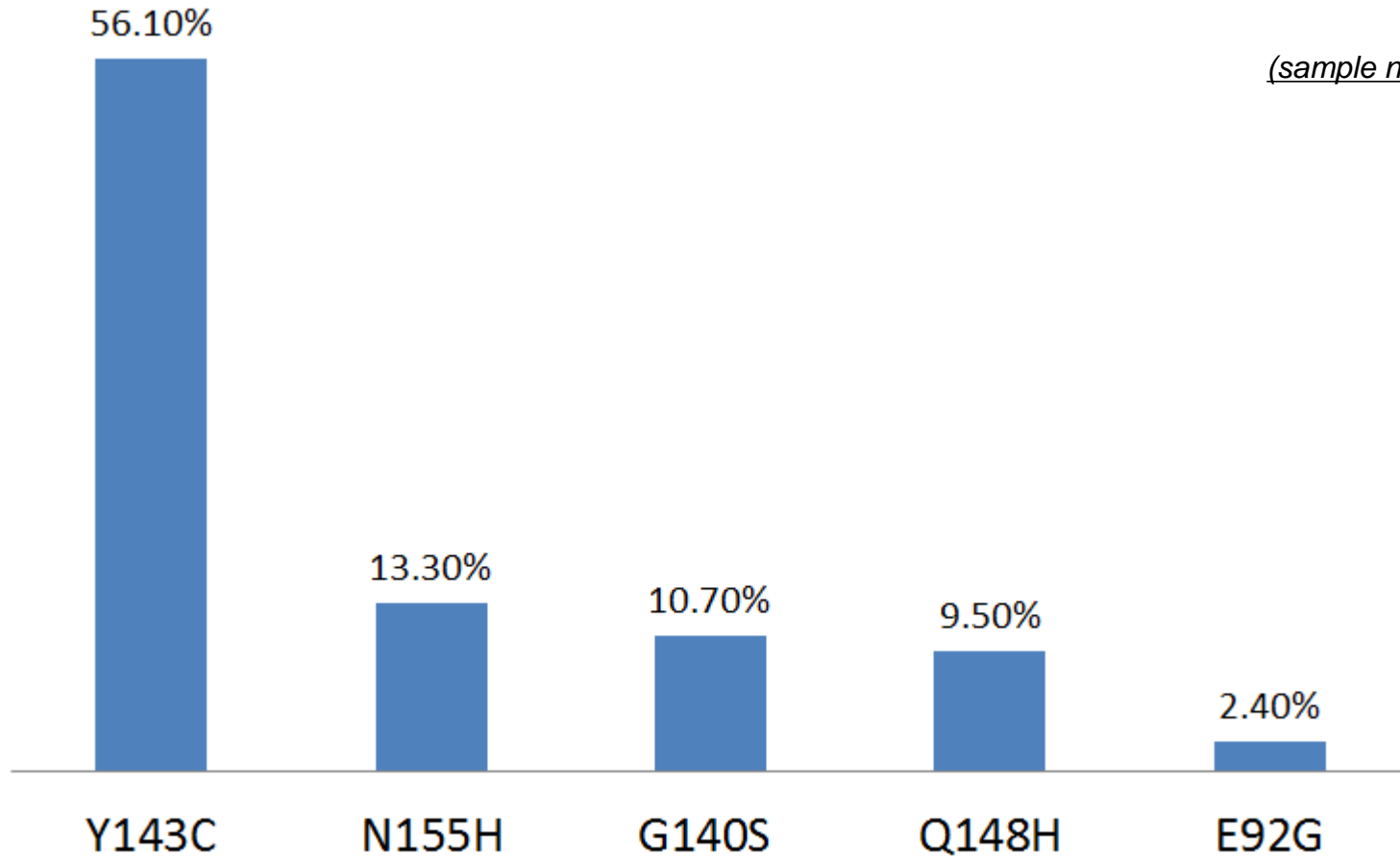
Integrase – found resistance mutations

- N = 27

| MUTATION | Sanger | 20% | 15% | 10% | 5% | 2% | 1% |
|---------------------|-----------------|-----------------|-----------------|------------------|------------------|------------------|------------------|
| N155H | 2 | 2 | 2 | 3 | 3 | 4 | 4 |
| Q148H | 1 | 1 | 1 | 2 | 2 | 2 | 2 |
| Q148R | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| E92Q | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| Y143C | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| S147G | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| T66A | 0 | 1 | 1 | 1 | 1 | 1 | 2 |
| <u>total</u> | <u>4</u> | <u>8</u> | <u>8</u> | <u>10</u> | <u>10</u> | <u>12</u> | <u>13</u> |

Raltegravir , Elvitegravir, Dolutegravir???

(sample not in data set)



| | | | | | |
|------------|-----------|------------|------------|------------|------------|
| RAL | 60 | 120 | 150 | 210 | 225 |
| EVG | 10 | 70 | 100 | 160 | 190 |
| DTG | 0 | 10 | 20 | 65 | 70 |

envelope / tropism

tropism predictions

- N = 97

Sanger

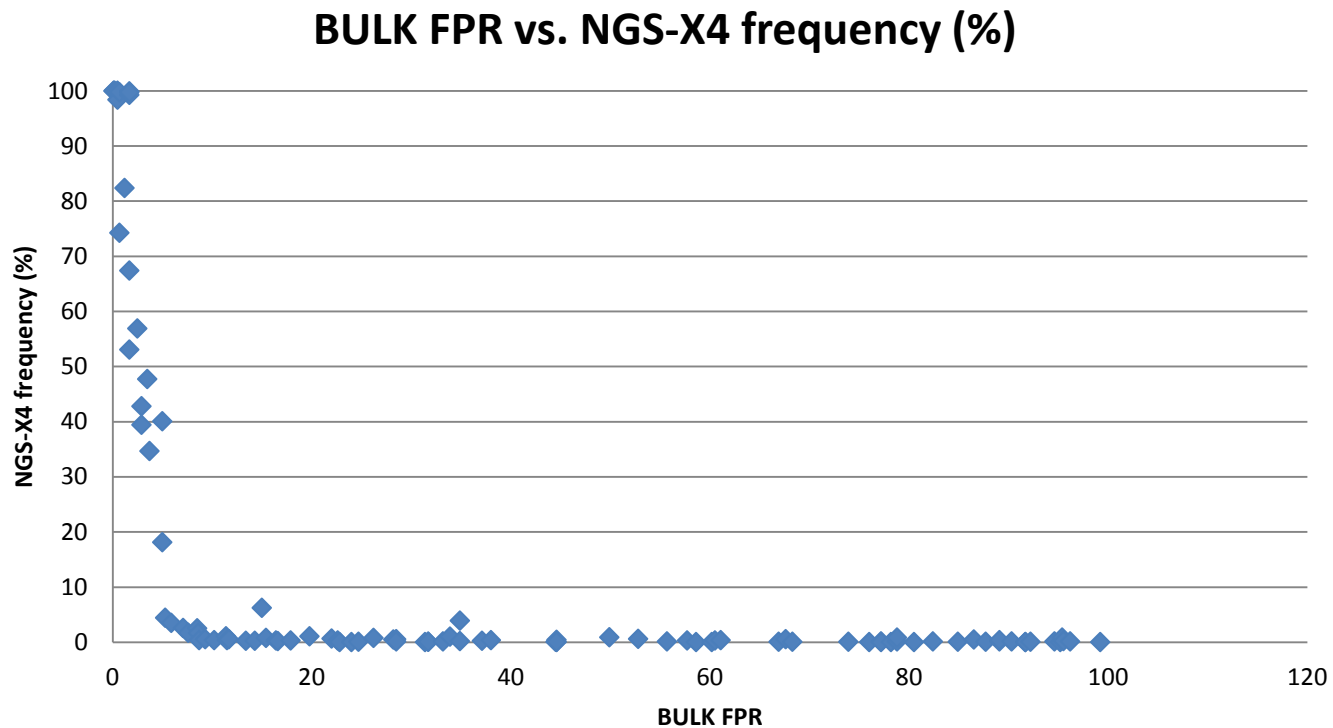
| | RED (<5%) | YELLOW | GREEN (>15%) |
|--------|-----------|--------|--------------|
| NGS R5 | 0 | 10 | 61 |
| NGS X4 | 18 | 7 | 1 |

| | 5-10% | 10-15% |
|--------|-------|--------|
| NGS R5 | 4 | 6 |
| NGS X4 | 6 | 1 |

| | <10% | >10% |
|--------|------|------|
| NGS R5 | 4 | 67 |
| NGS X4 | 24 | 2 |

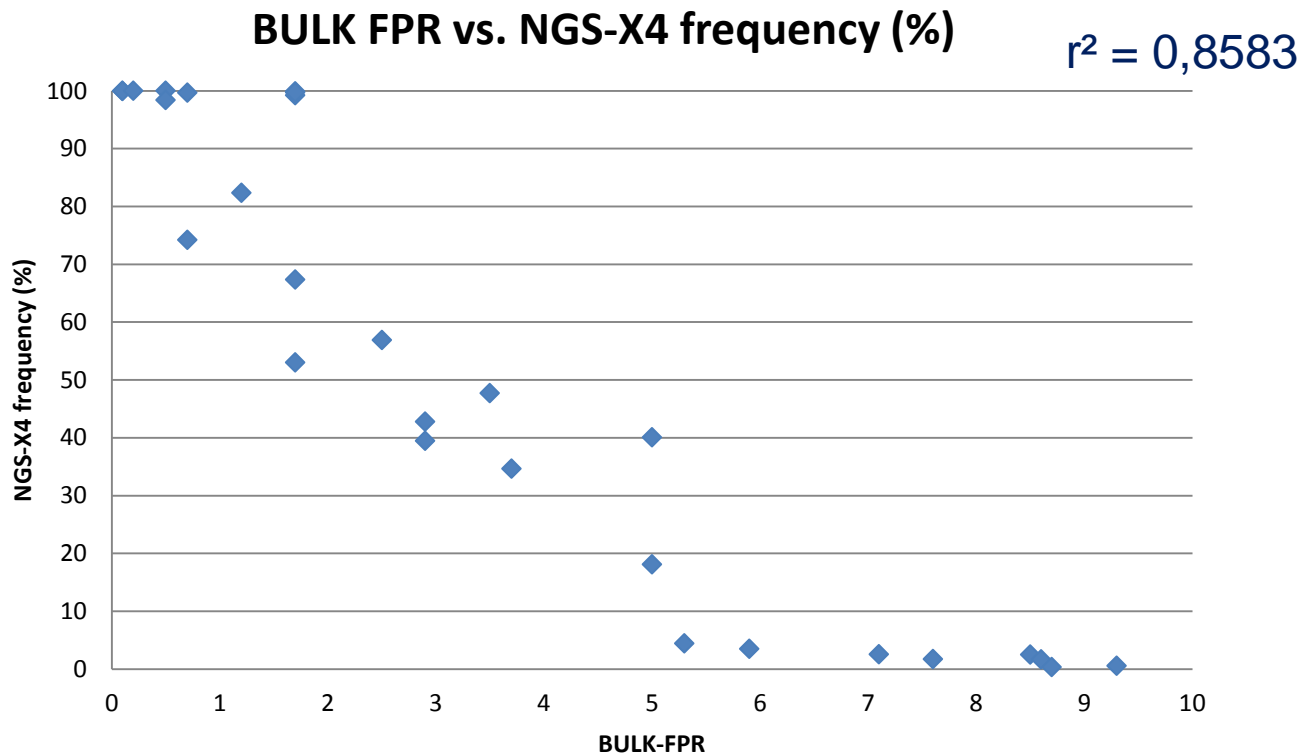
tropism predictions

- N = 97



tropism predictions

- N = 97



conclusions

- NGS technologies can be used in the daily routine
- altogether: limit of detection of Sanger sequencing between 15% and 20%
- but: Sanger sequencing seems to be
 - more sensitive in case of NNRTI mutations
 - less sensitive in case of PI and II mutations
 - dependent on the background / surrounding positions
- Meaningful clinical cutoffs still to be determined
- NGS X4 frequencies correlate with FPRs of <10%

Acknowledgments



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