

## Examples of sub-optimal library profiles for HiSeq runs

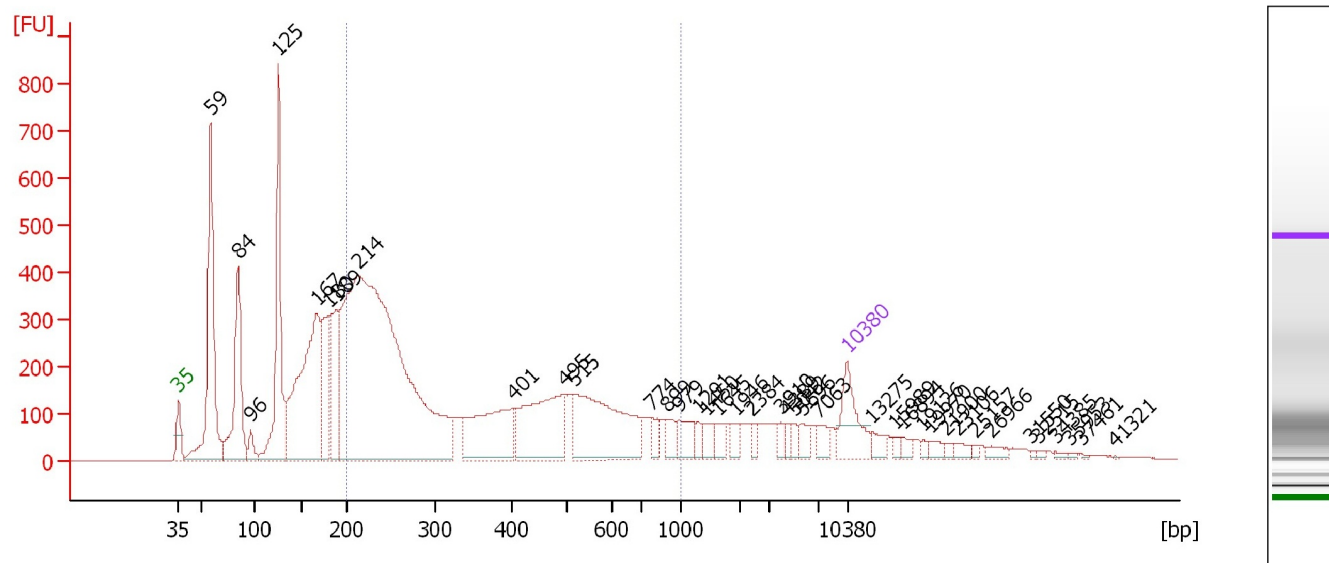
General features of *sub-optimal* library profiles on the high-sensitivity DNA chip:

- multiple peaks
- primers present (peaks up to 70 bp)
- empty library present
  - Standard Illumina: peak at ~125-130 bp
  - SREK: peaks at 80 and 90 bp
  - SAGE: peak at 85 bp
- broad peak size distribution
- library insert size too short for the run type
- concentration too low
- no peak(s) at all

### Illumina library

Multiple problems:

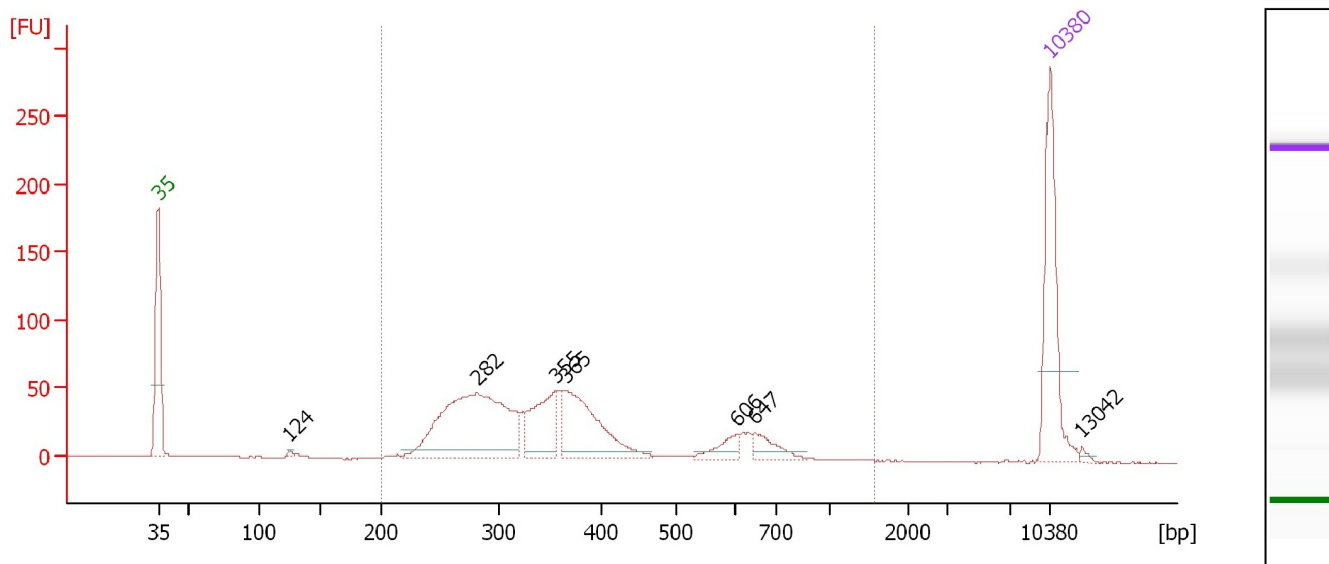
- primers present (59 and 84 bp)
- empty library (125bp)
- broad size distribution
- most prominent 'peak' at ~210 bp is too short for a 2x100 PE run
- **never sequence this**
- **re-prep these samples!**



# Examples of sub-optimal library profiles for HiSeq runs

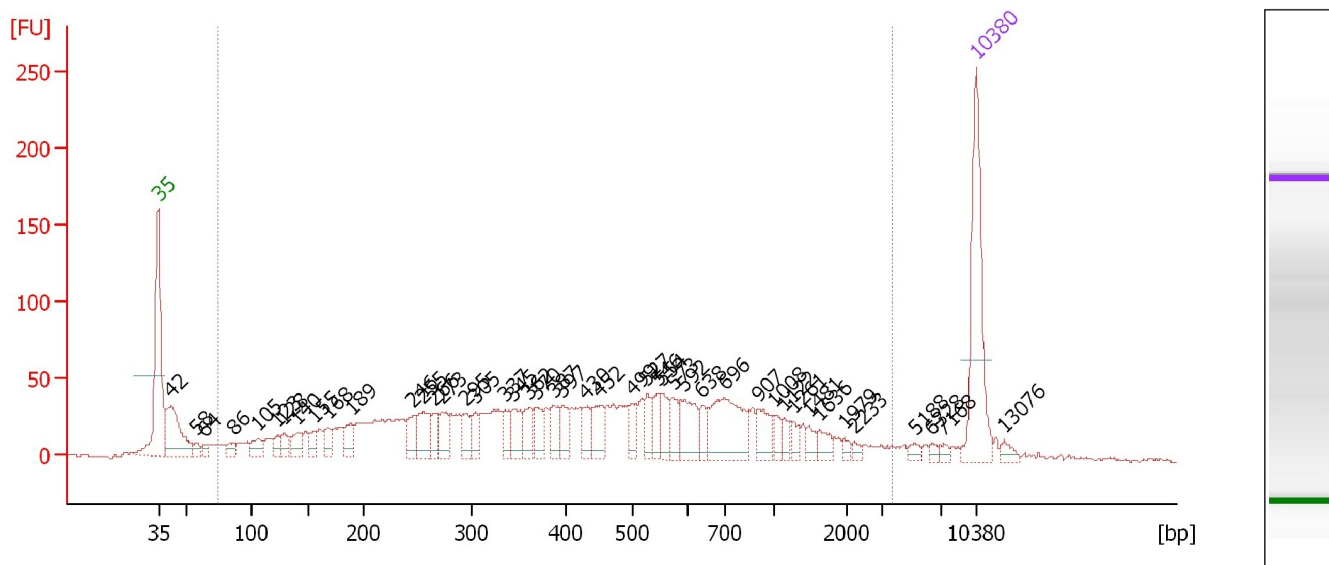
## Illumina library

problem: Multiple peaks visible at ~280, ~360 and ~650 bp (and 124bp). This will result in a lower number of data points and lower quality reads due to large variations in cluster size.  
 → **size select the 360 bp library or re-prep the sample**



## Illumina library

problem: no real peak to see.  
 → **never sequence this**  
 → **size select the 360 bp library or re-prep the sample!**



## Examples of sub-optimal library profiles for HiSeq runs

### Illumina library

problem: primers present at 59 and 89 bp. These will occupy the oligos on the Illumina flowcell and impair cluster formation

**2x100 PE run:**

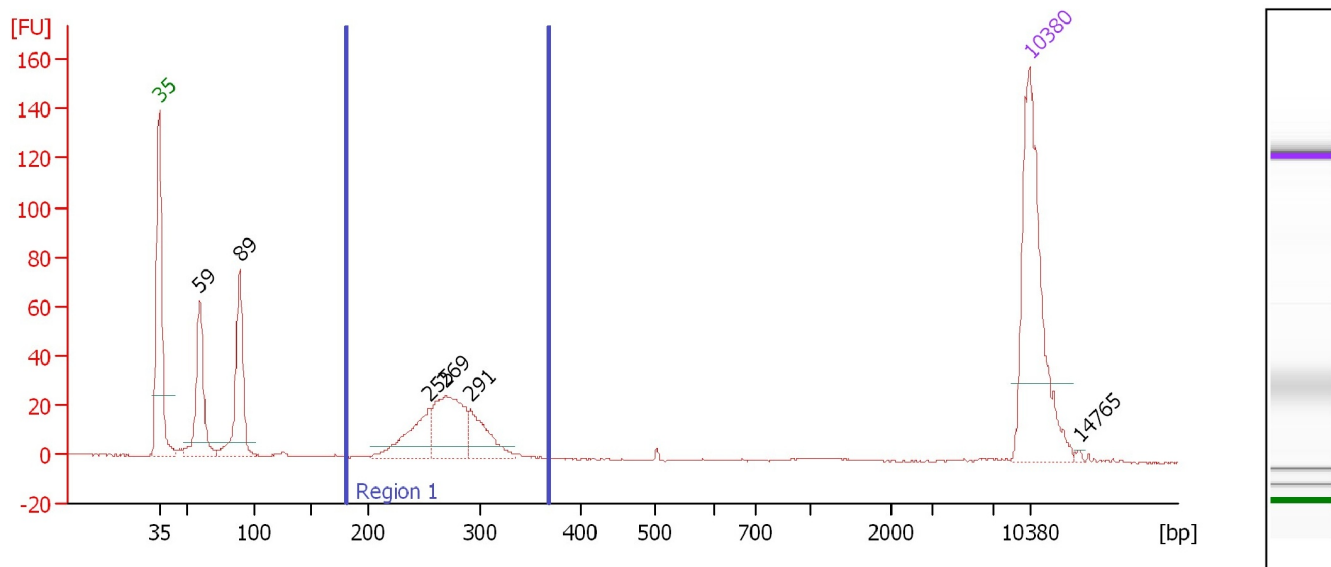
**library insert size is too short**

→ re-prep the sample

**50 SE run:**

**library insert size is OK!**

→ re-size select the library



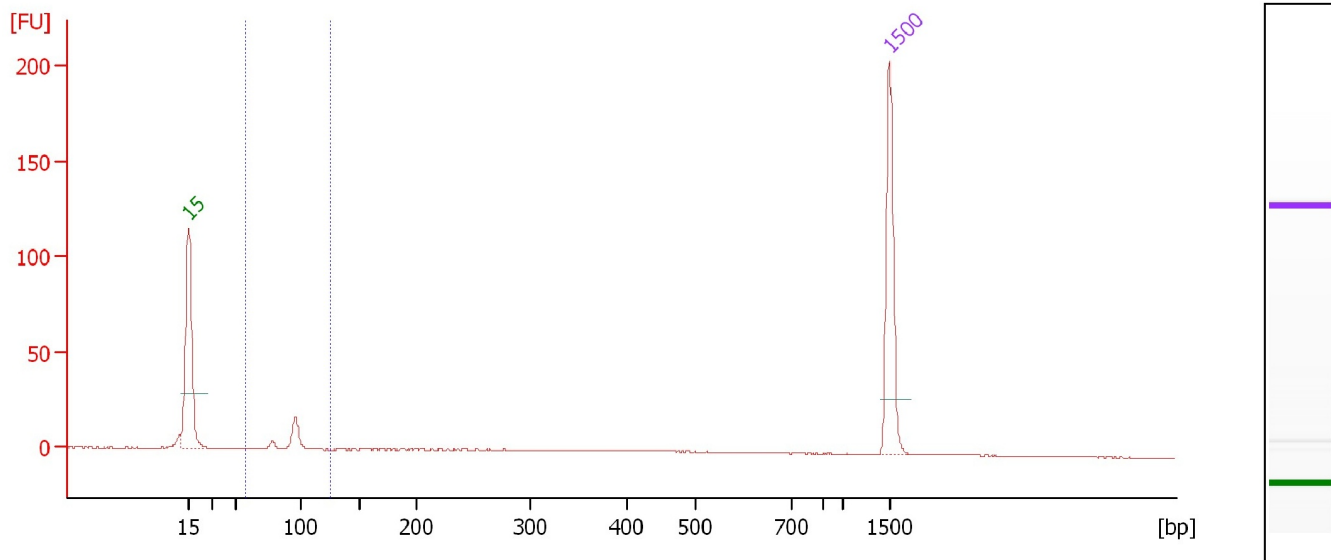
### SAGE library

problem:

- additional peak at ~85 bp

→ re-size select the library or

→ re-prep these samples!

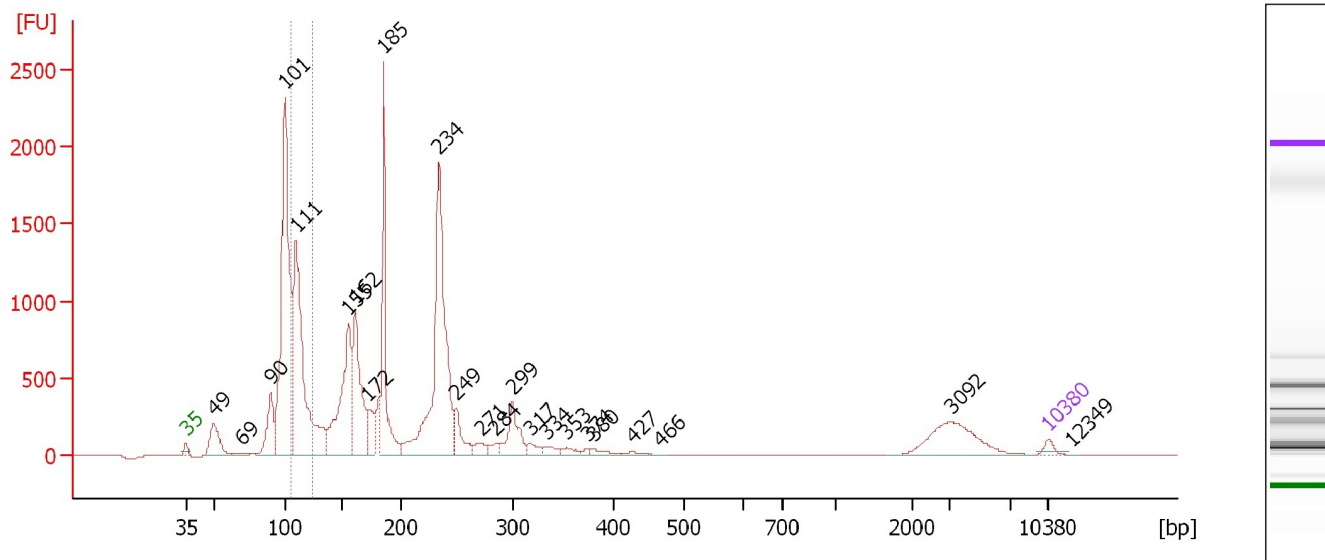


# Examples of sub-optimal library profiles for HiSeq runs

## SREK library (miRNA)

problem: Multiple problems:

- primers present (49 and 69 bp)
  - empty library (90bp)
  - additional long insert fragments
- **re-size select the library or**  
 → **re-prep these samples!**

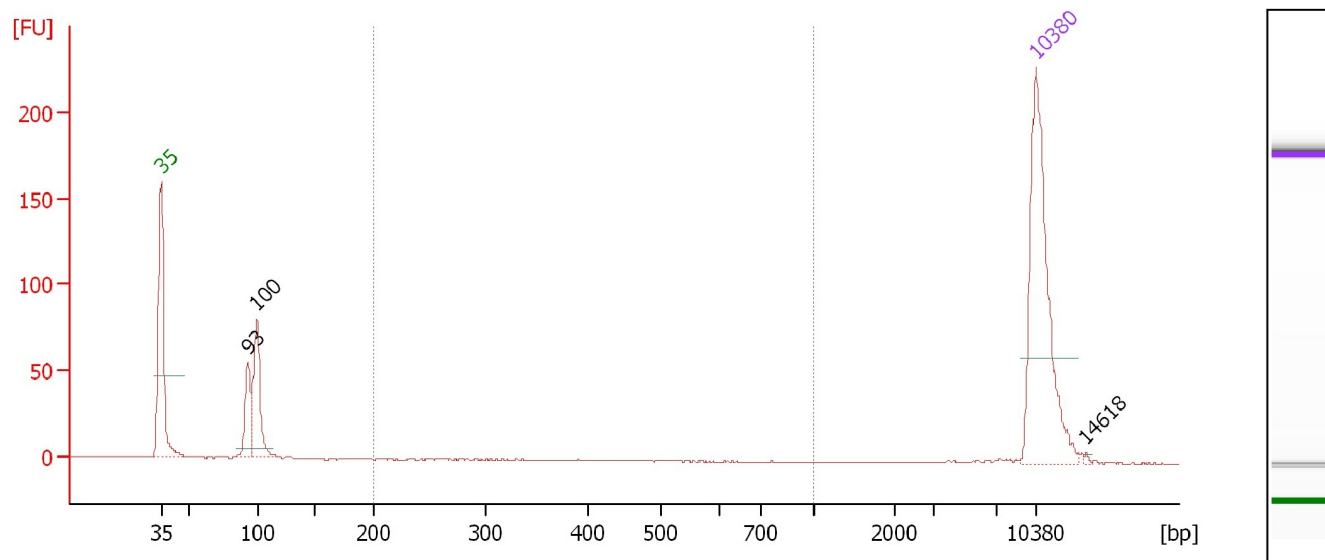


## SREK library (miRNA)

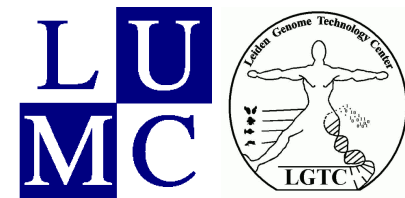
problem: Short library fragment present.

The shorter peak at 93bp is an artefact that can be sequenced, but does not result in relevant data.

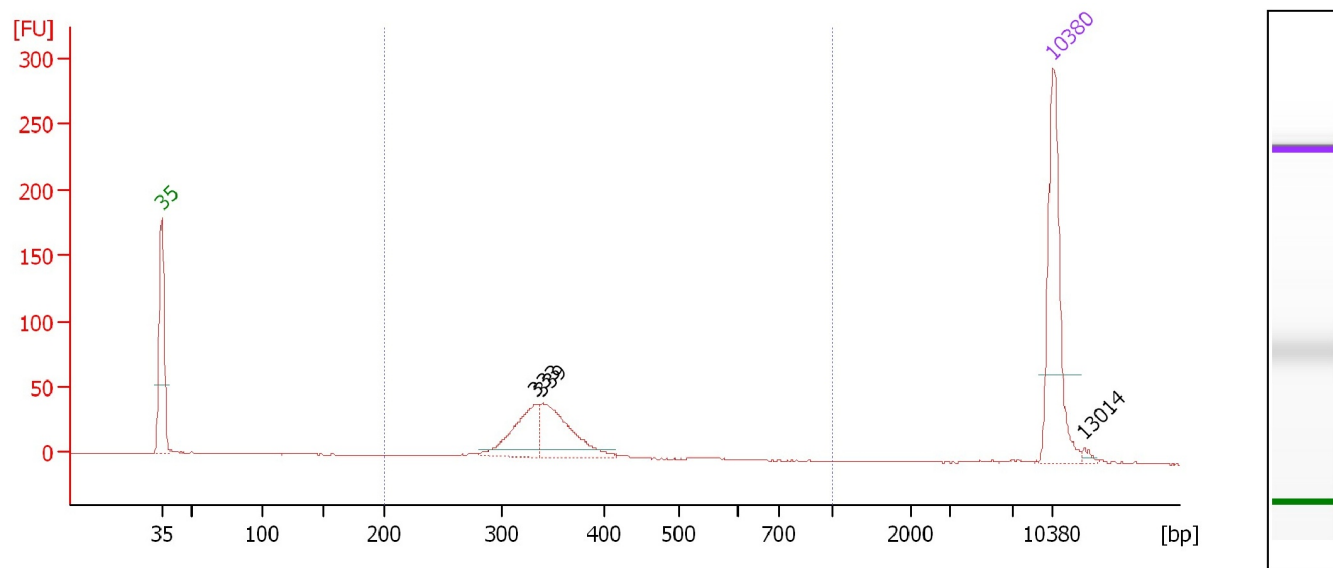
- **re-size select the library or**
- **re-prep these samples!**



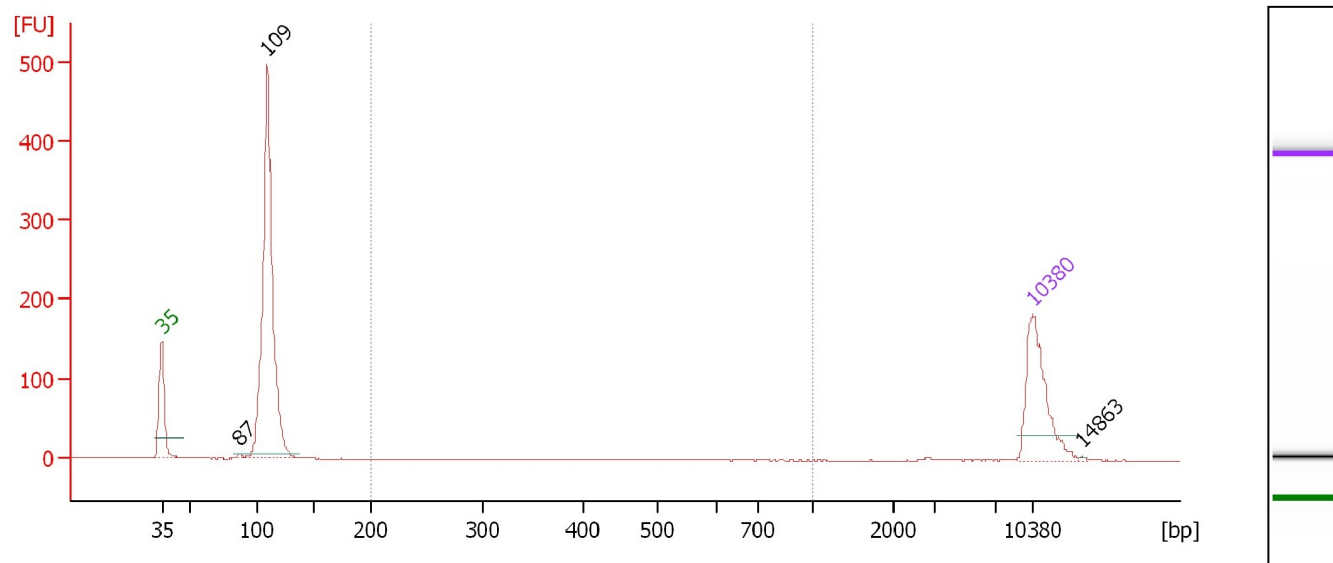
## Examples of high quality library profiles for HiSeq runs

**Illumina library**

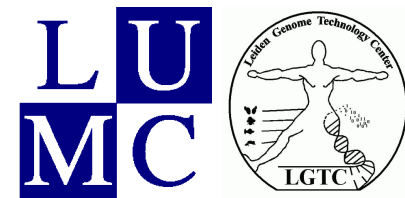
Singular peak at ~350 bp, no primers or empty library fragments present!  
 Good library size for a 2x100-PE run  
 Peak range of 350-600 bp is OK!

**SREK library**

Singular peak at ~109 bp, no primers or high abundant empty library fragments present!

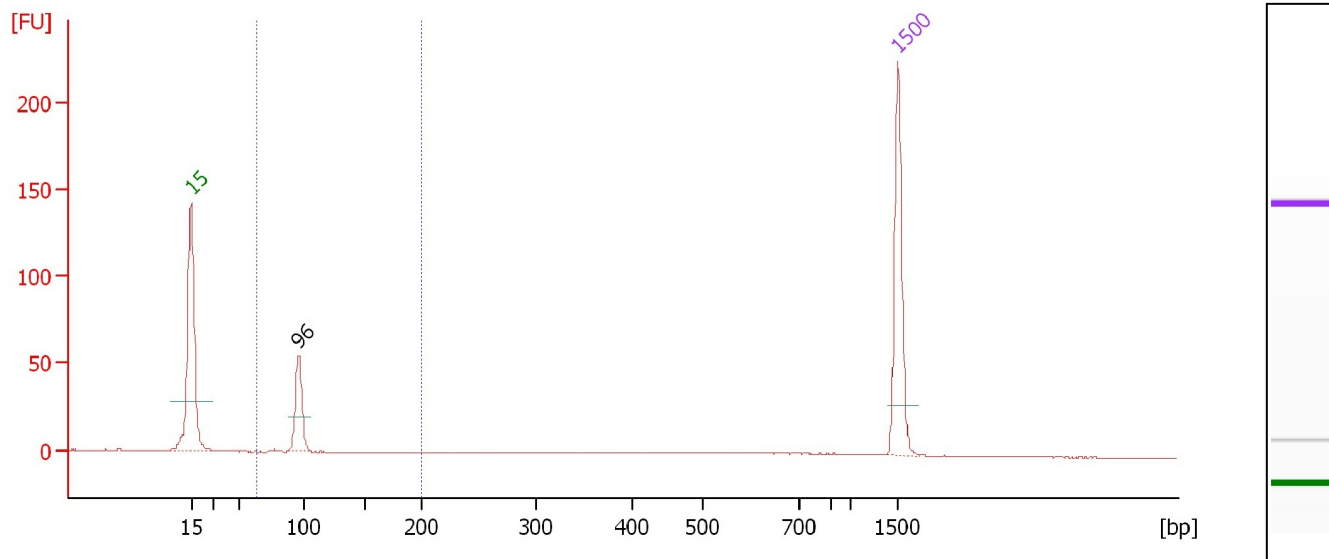


## Examples of high quality library profiles for HiSeq runs



### SAGE library

Singular peak at ~96 bp, no primers or empty library fragments present!



When in doubt, contact the LGTC team

After successful sample prep, fill in the forms at the LGTC Lims3 system:

<http://10.160.8.32/lims3/> (login = lgtc; pass = lgtc)

**!! We cannot sequence samples that are not in our Lims system !!**