RNA society meeting 2022

circRNA detection tool benchmarking

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CRIG
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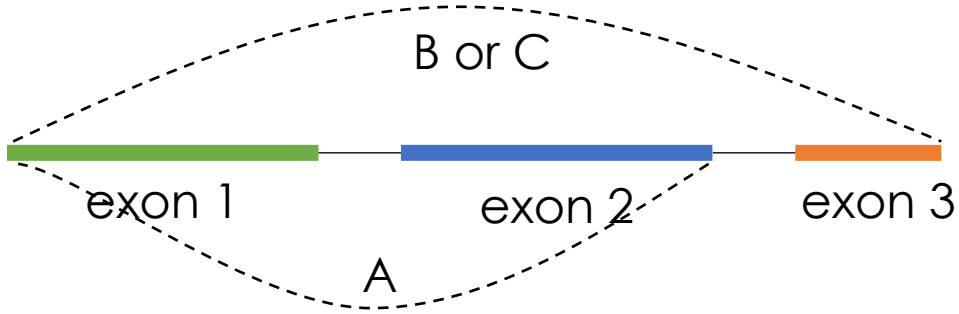
OncoRNALab

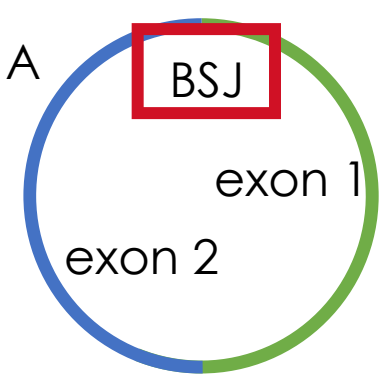
Stichting tegen Kanker

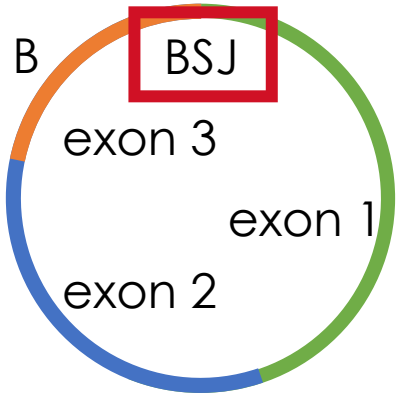
circular RNAs are formed through back-splicing

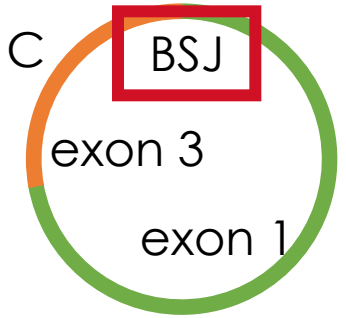
unique circRNA feature: backsplice junction (BSJ)



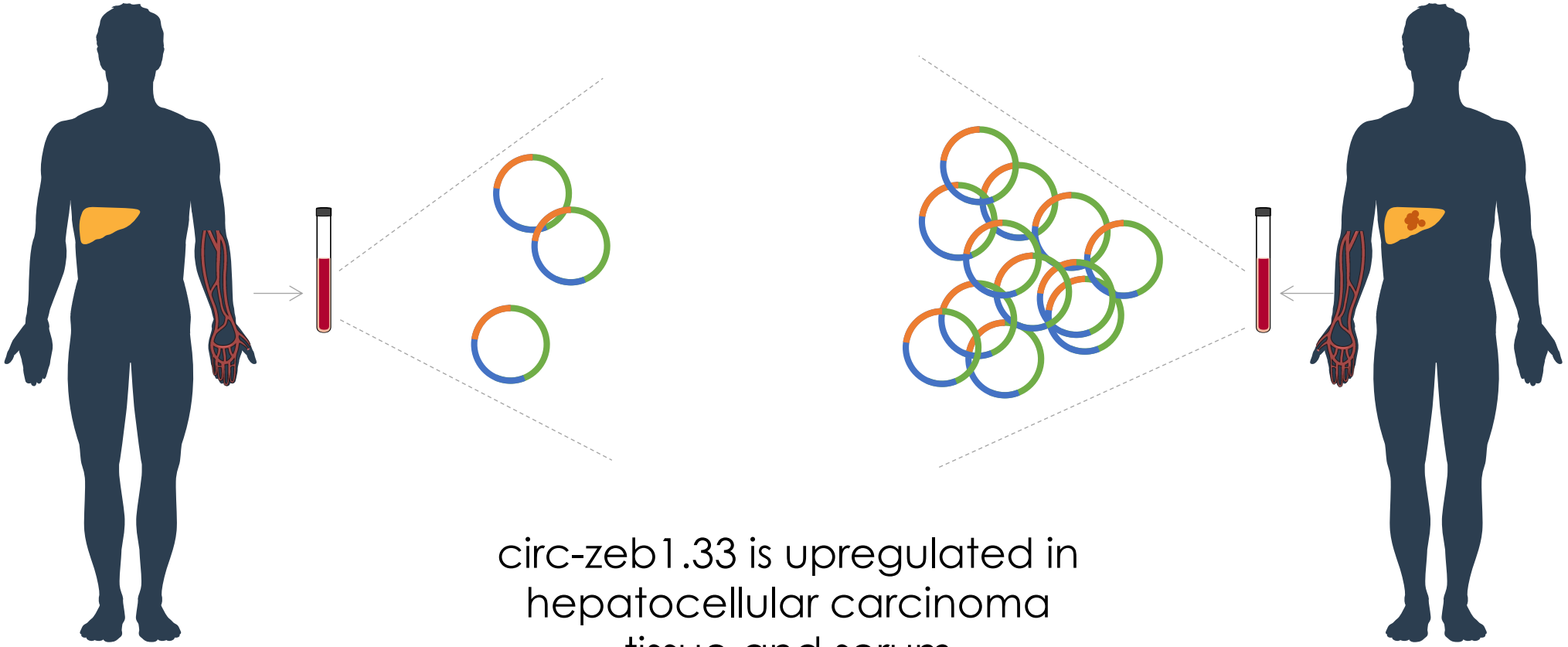
backsplicing







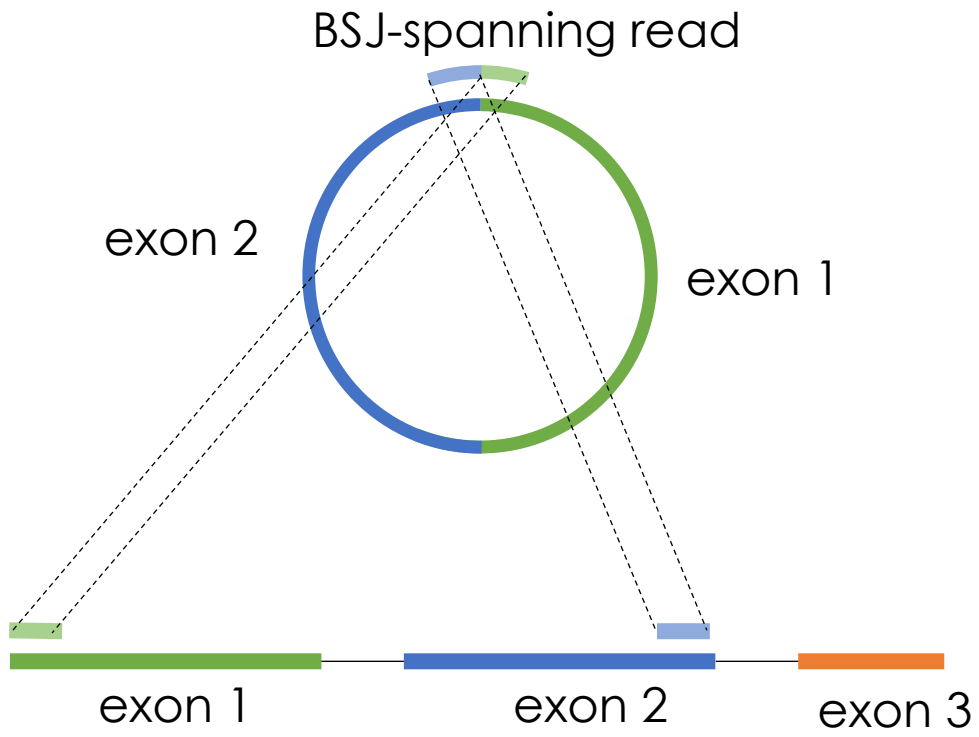
circRNAs have the potential to serve as cancer biomarkers



circ-zeb1.33 is upregulated in hepatocellular carcinoma tissue and serum

circRNA detection based on RNA sequencing data

- step 1: mapping
- step 2: circRNA detection in unmapped reads



which algorithm to use for circRNA detection?

- > 40 circRNA prediction tools ⇒ lack of extensive orthogonal validation
- differences in
  - mapping tool
  - candidate-based VS segmented read-based approach
  - filtering
  - …

CirComPara: A Multi-Method Comparative Bioinformatics Pipeline to Detect and Study circRNAs from RNA-seq Data

Enrico Gaffo [1], Annagiulia Bonizzato [2], Geertruy te Kronnie [2] and Stefania Bortoluzzi [1,*]

Circular RNA profile in gliomas revealed by identification tool UROBORUS

Xiaofeng Song[1], Naibo Zhang[2], Ping Han[3], Byoung-San Moon[2], Rose K. Lai[4,*], Kai Wang[5,*] and Wange Lu[2,*]

Acfs: accurate circRNA identification and quantification from RNA-Seq data

Xintian You[1] & Tim OF Conrad[1,2]

RESEARCH Open Access

Statistically based splicing detection reveals
nt and tissue-specific inducti
luring human fetal developn

han J. Palpant[3], Peter L. Wang[1], Nastaran Afari[2], Chuan Jia
nt[2*] and Julia Salzman[1*]

METHOD Open Access

A multi-split mapping algorithm for circular RNA, splicing, trans-splicing and fusion detection

Steve Hoffmann[1,2,3], Christian Otto[1,2,3], Gero Doose[1,2,3], Andrea Tanzer[4], David Langenberger[1,2,3], Sabina Christ[5], Manfred Kunz[6], Lesca M Holdt[3,7], Daniel Teupser[3,7], Jörg Hackermüller[2,5,8] and Peter F Stadler[1,2,3,4,9,10,11*]

Resource

Diverse alternative back-splicing and alternative splicing landscape of circular RNAs

METHOD Open Access

CIRI: an efficient and unbiased algorithm for *de novo* circular RNA identification

Yuan Gao[1,2†], Jinfeng Wang[1†] and Fangqing Zhao[1*]

Gene expression

circtools—a one-stop software solution for circular RNA research

Tobias Jakobi[1,2,*], Alexey Uvarovskii[1,2] and Christoph Dieterich[1,2,*]

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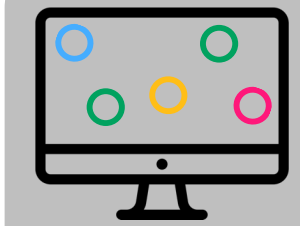
large-scale collaborative RT-qPCR benchmarking study of circRNA prediction tools

cancer
cell lines
(HLF, NCI-H23,
SW480)

deep paired-end
total RNA sequencing
(270-320 M reads)

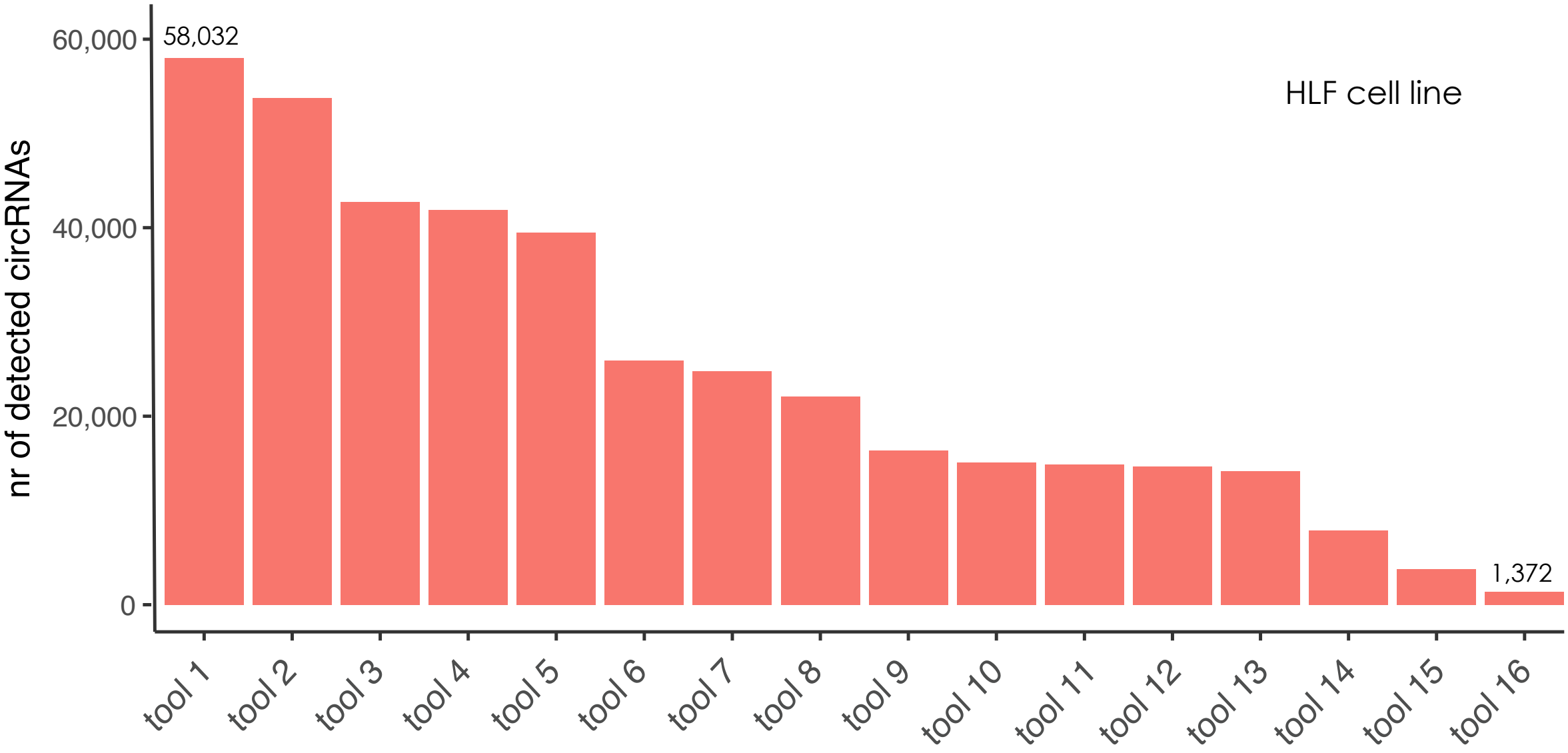


circRNA
detection by
collaborators
(n = 16)

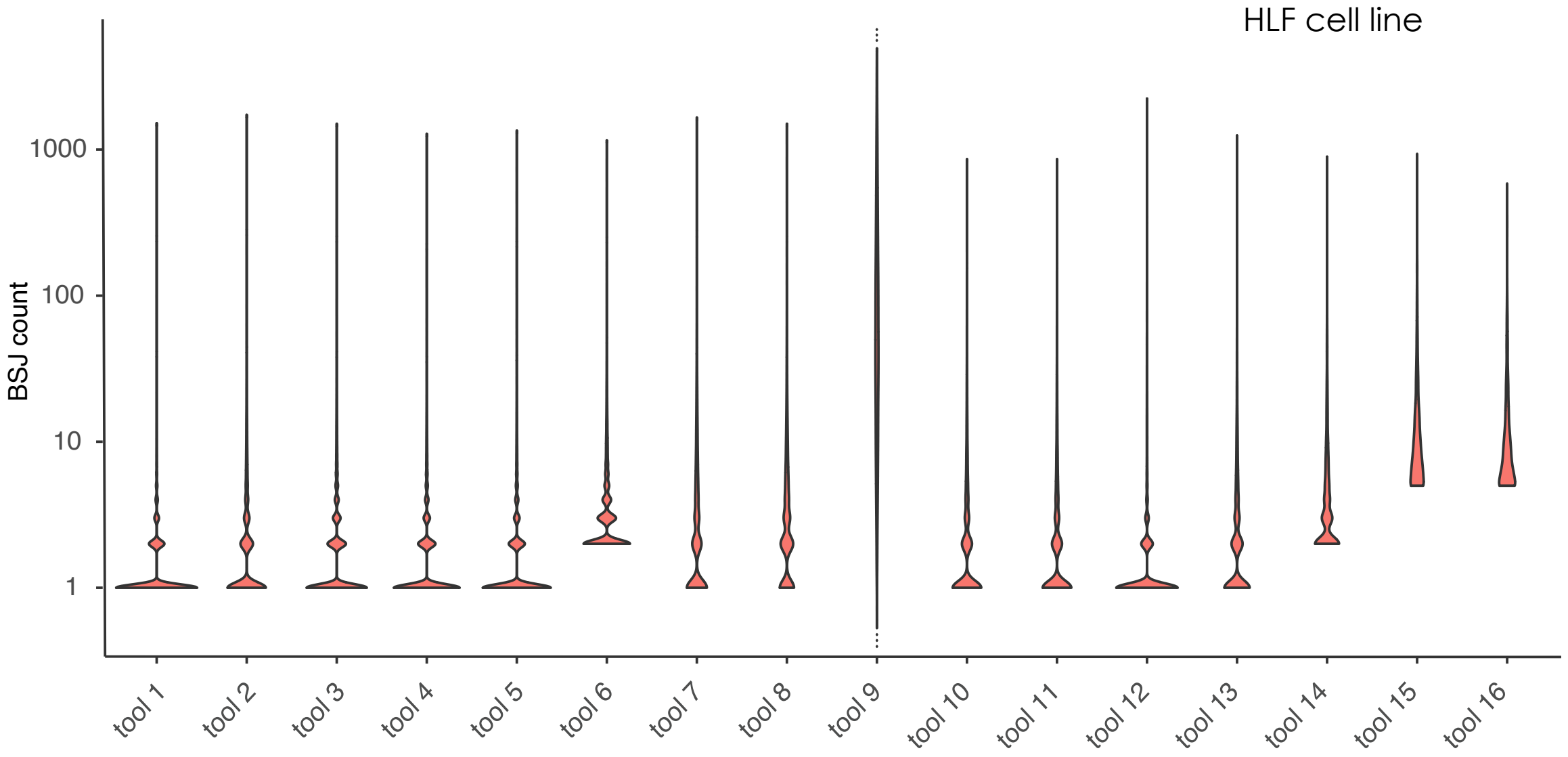
circRNA
selection

RT-qPCR and
RNase R validation
of predicted
circRNAs

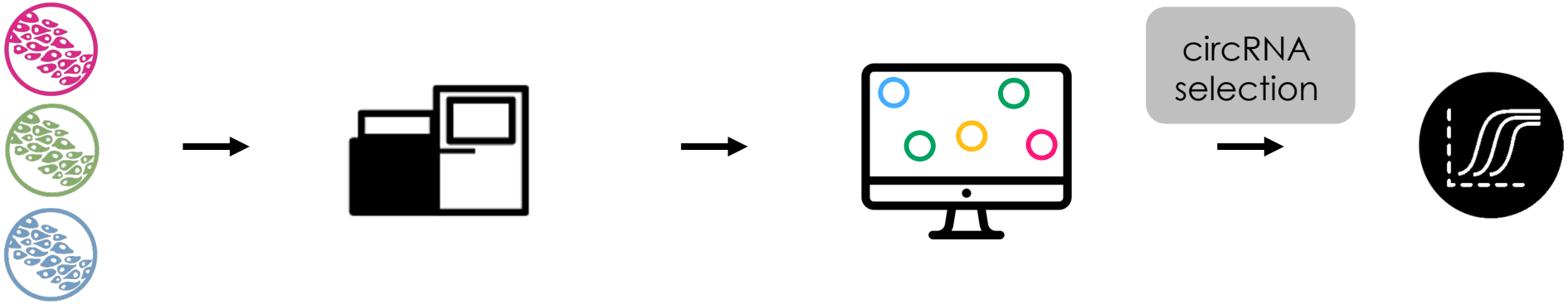
different tools predict a 40-fold difference in nr of circRNAs



different circRNA count distributions and filtering for different tools



large-scale collaborative RT-qPCR benchmarking study of circRNA prediction tools



cancer
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RT-qPCR and
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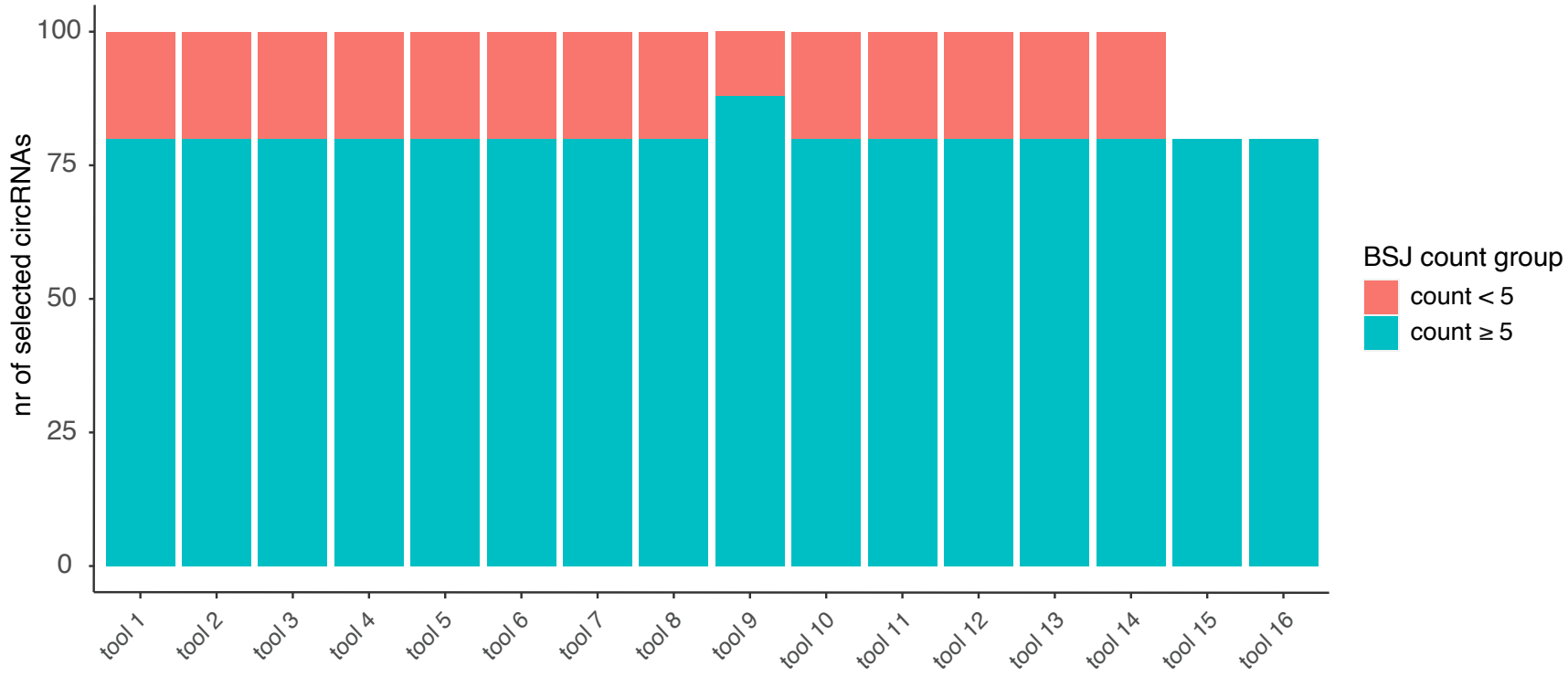
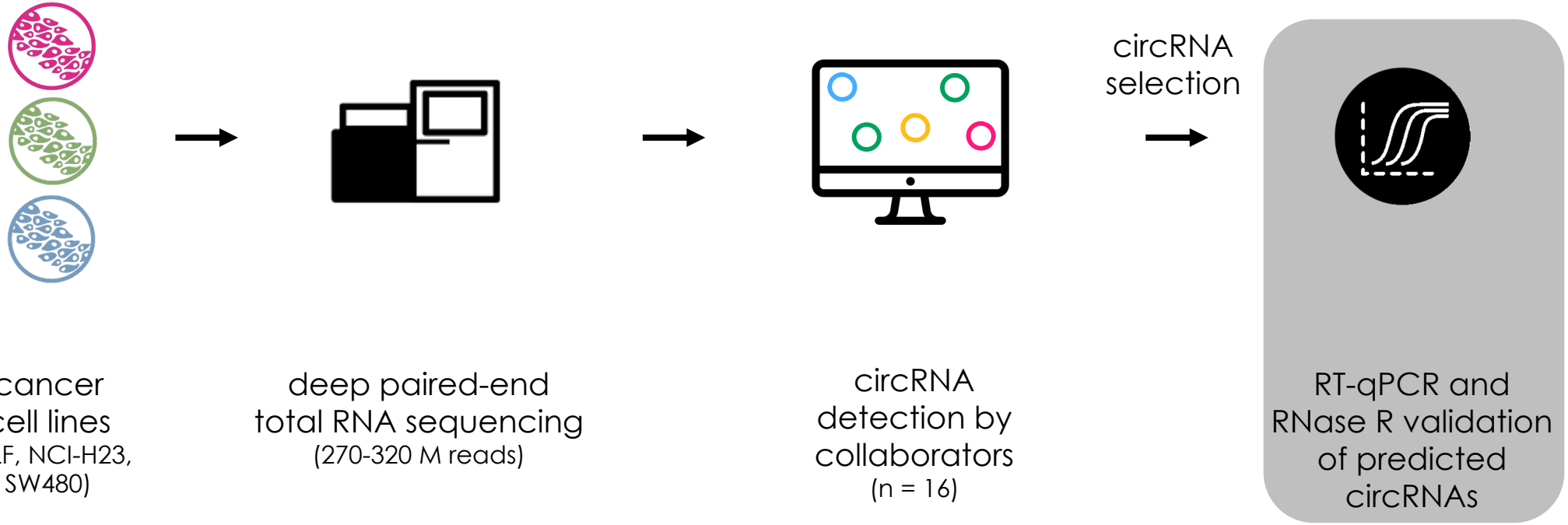
circRNA selection

- random selection of 100 circRNAs per tool
  - 20 circRNAs with BSJ count < 5
  - 80 circRNAs with BSJ count ≥ 5
- 1560 seleted circRNAs

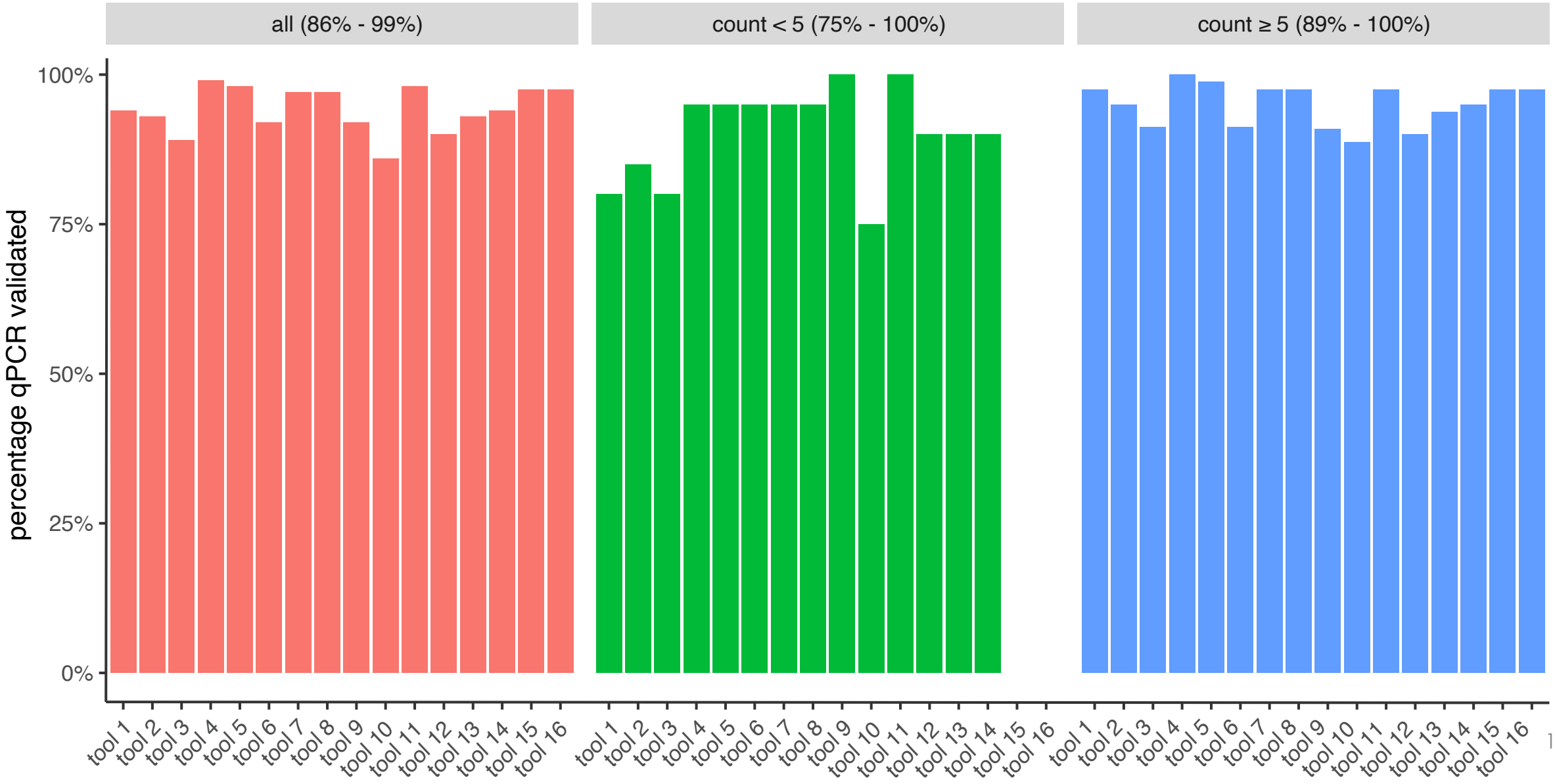
tool 9 does not report raw BSJ counts, but only reports TPMs
⇒ 100 circRNAs were choosen randomly



large-scale collaborative RT-qPCR benchmarking study of circRNA prediction tools

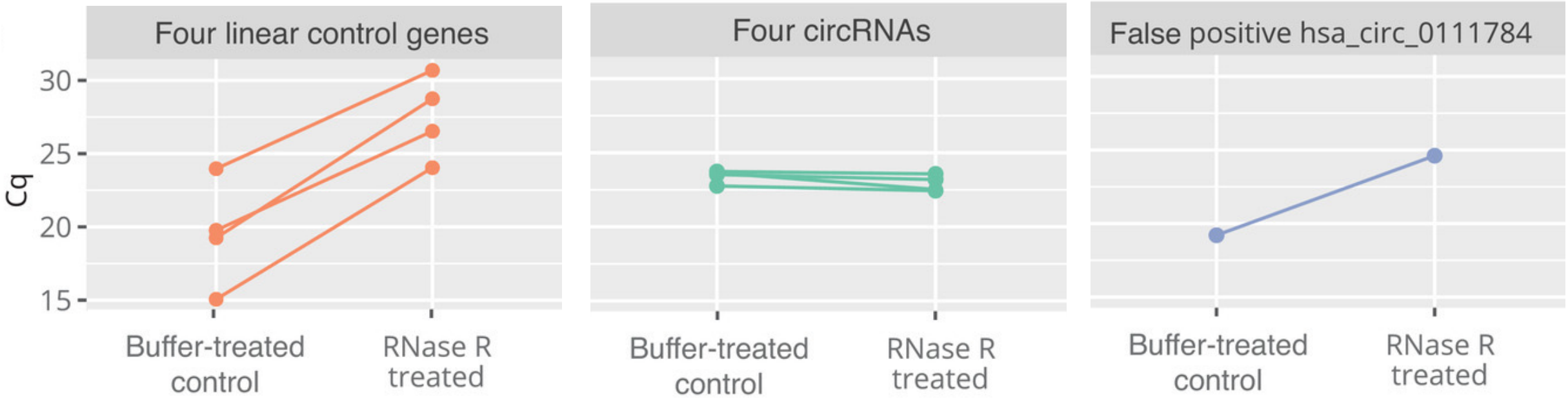


step 1: qPCR validation



step 2: RNase R validation

- validate that the qPCR target is a true circRNA, and not linear RNA
- RNase R degrades linear RNAs
- validation = $Cq_{treated} - Cq_{untreated} > 3$



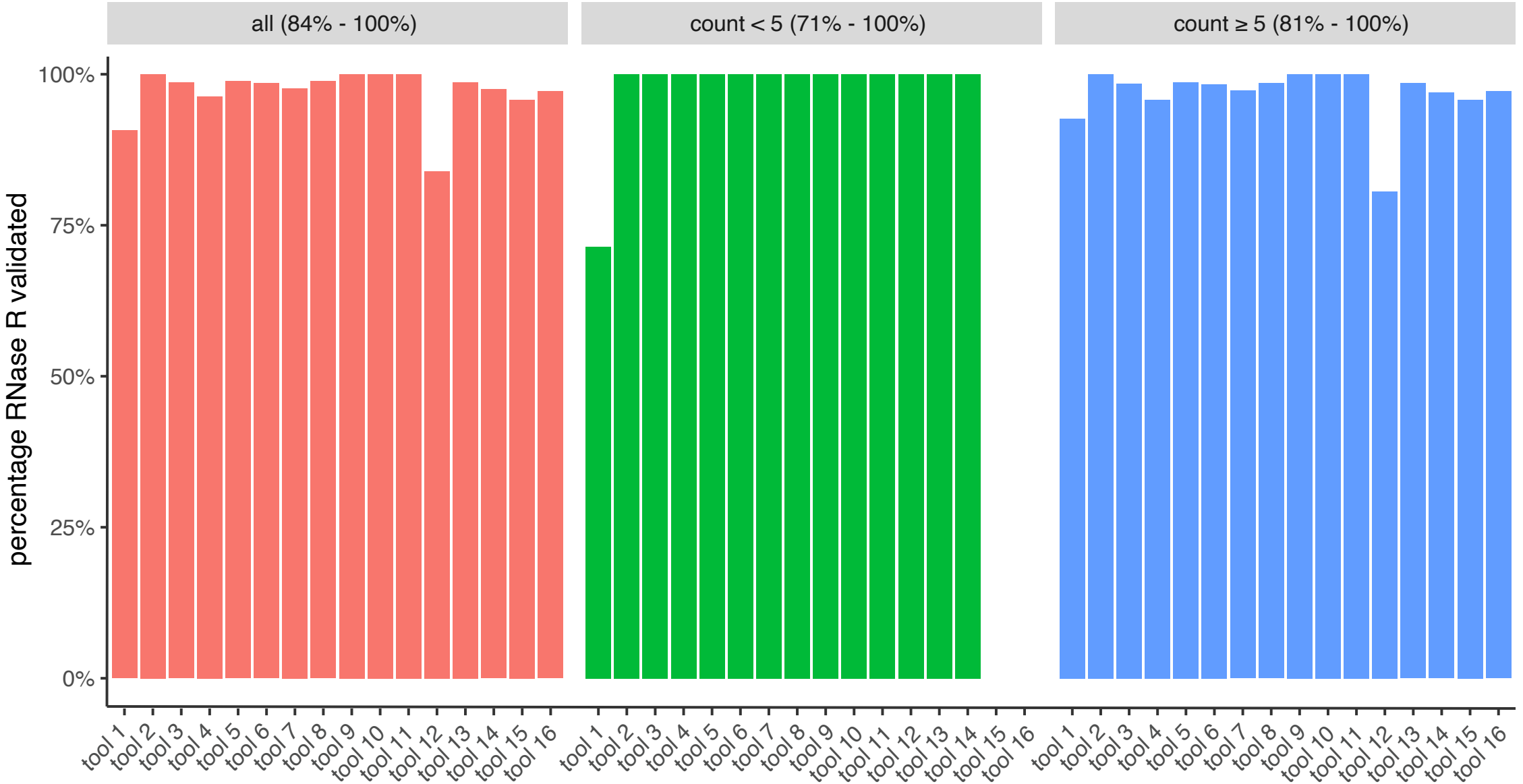
Cq increase of 8.17 (SD = 1.42)

Cq increase of 0.49 (SD = 0.43)

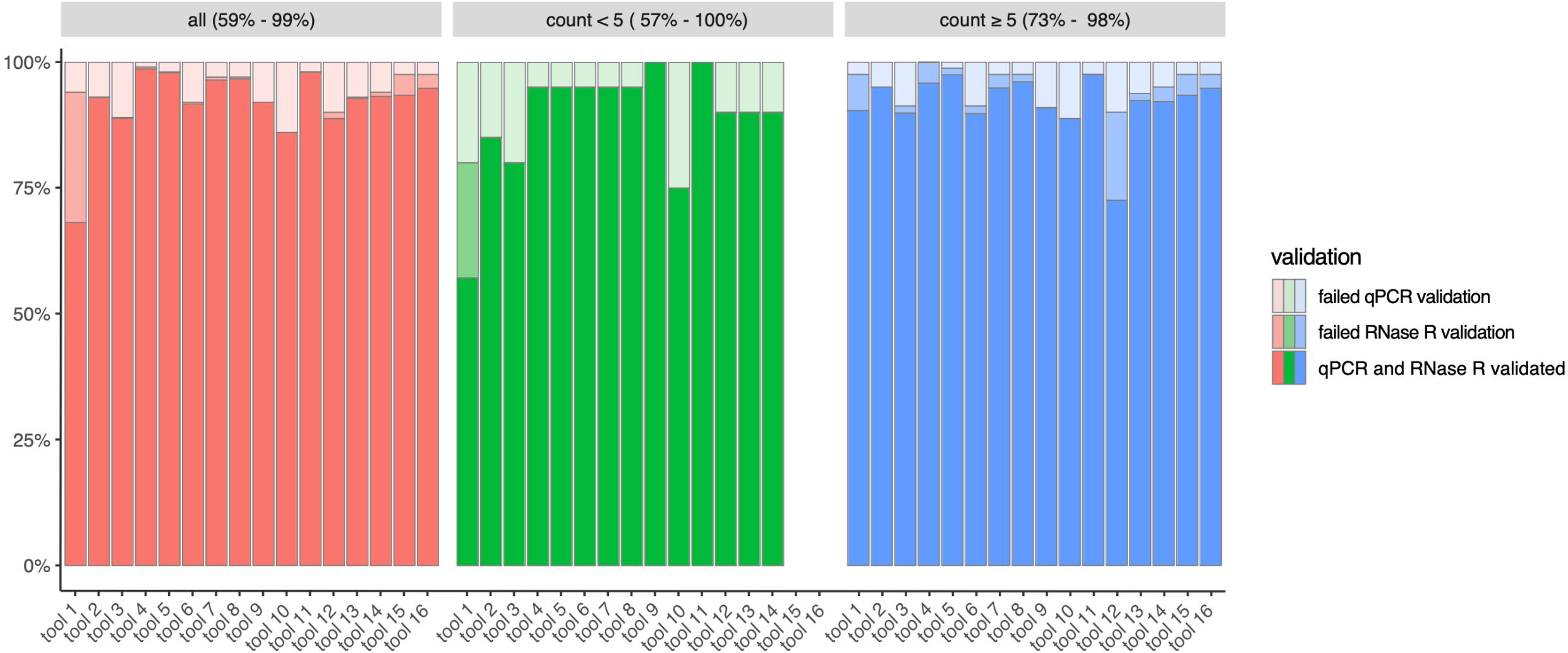
Cq increase of 5.3

step 2: RNase R validation

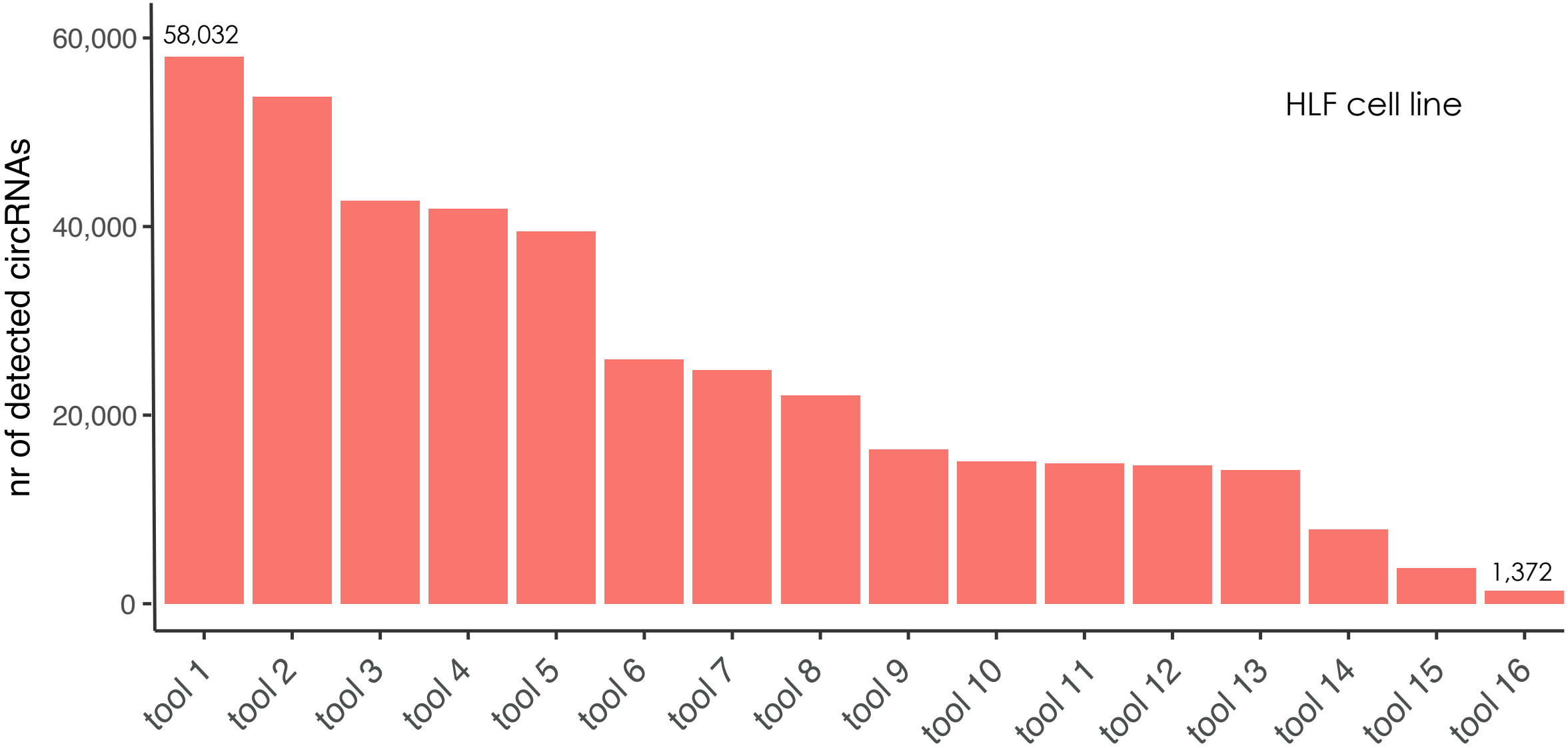
validation = $Cq_{treated} - Cq_{untreated} > 3$



combination of qPCR and RNase R validation rates

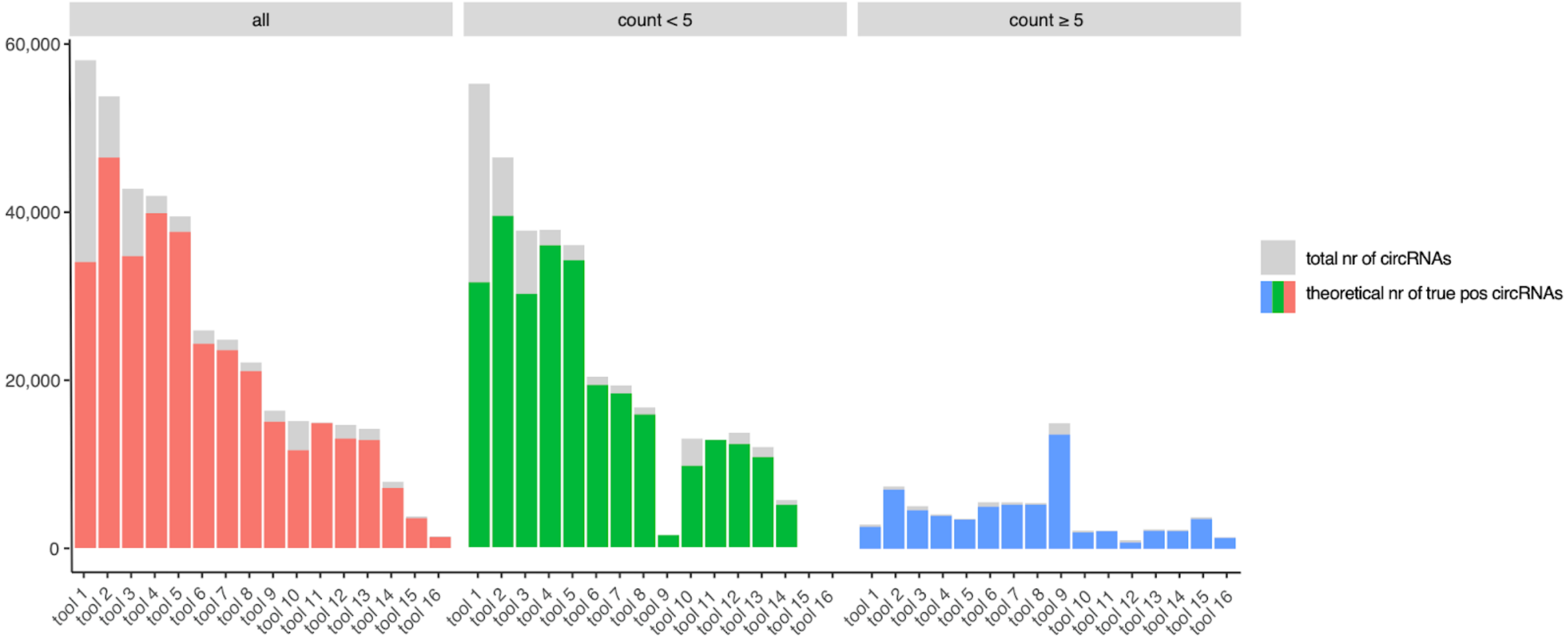


going back to the first slide



extrapolation to total nr of predicted circRNAs

theoretical nr of true positive circRNAs = total nr of detected circRNAs * combined validation rate



conclusions

- work in progress
  - amplicon sequencing data-analysis
  - overlap between tools
  - combination of tools

- circRNA detection tools generate vastly different numbers of circRNAs
- RT-qPCR and RNase R validation rates are generally quite good

OncoRNALab

Jasper Anckaert
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Jo Vandesompele
Kimberly Verniers
Pieter-Jan Volders
Nurten Yigit

Thank you!

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