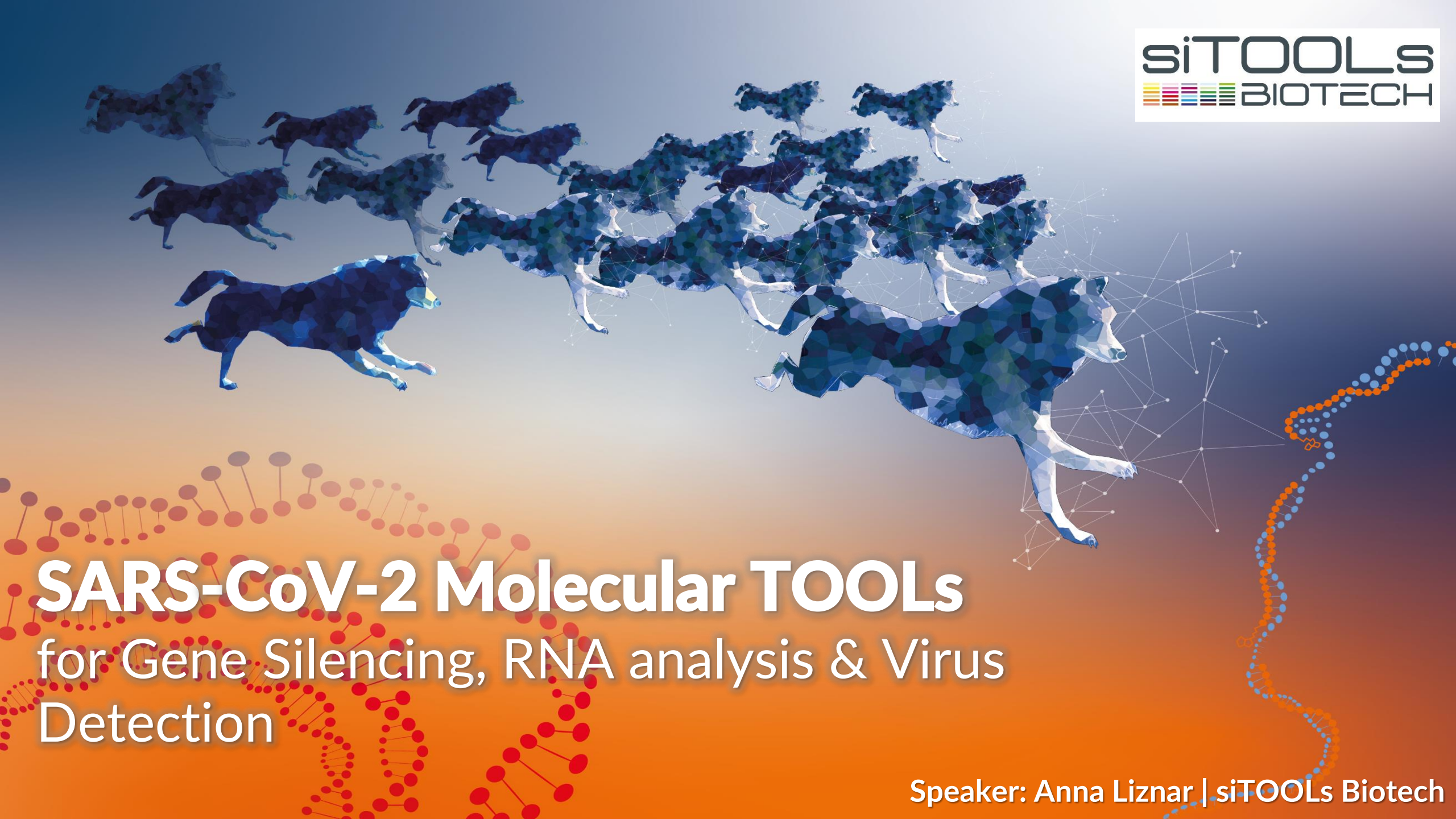


SARS-CoV-2 Molecular TOOLs

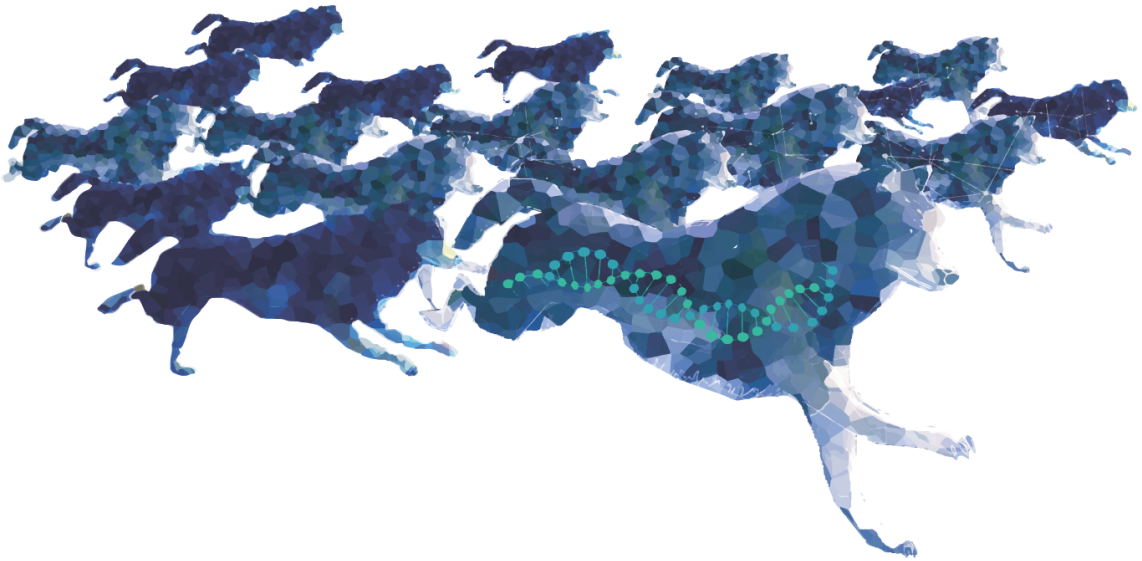
for Gene Silencing, RNA analysis & Virus Detection

Speaker: Anna Liznar | siTOOLs Biotech

About siTOOLs Biotech

- Founded in 2013
- Spin-out of **Intana Bioscience** and **University of Regensburg**
- All Scientist Team
- **Our Products:** highly complex, optimally designed oligonucleotide pools
- **Our Services:** Big data analysis (RNAi & CRISPR), RNAi screening/expression analysis projects

Overview - Product



siPOOLs

- Complex siRNA pools for rapid & reliable gene silencing
- For target identification and target validation

riboPOOLs

- Complex pooled biotinylated ssDNA oligos
- Depletion of ribosomal RNA (rRNA)

raPOOLs

- complex pooled biotinyated ssDNA oligos
- For robust & targeted RNA Capture

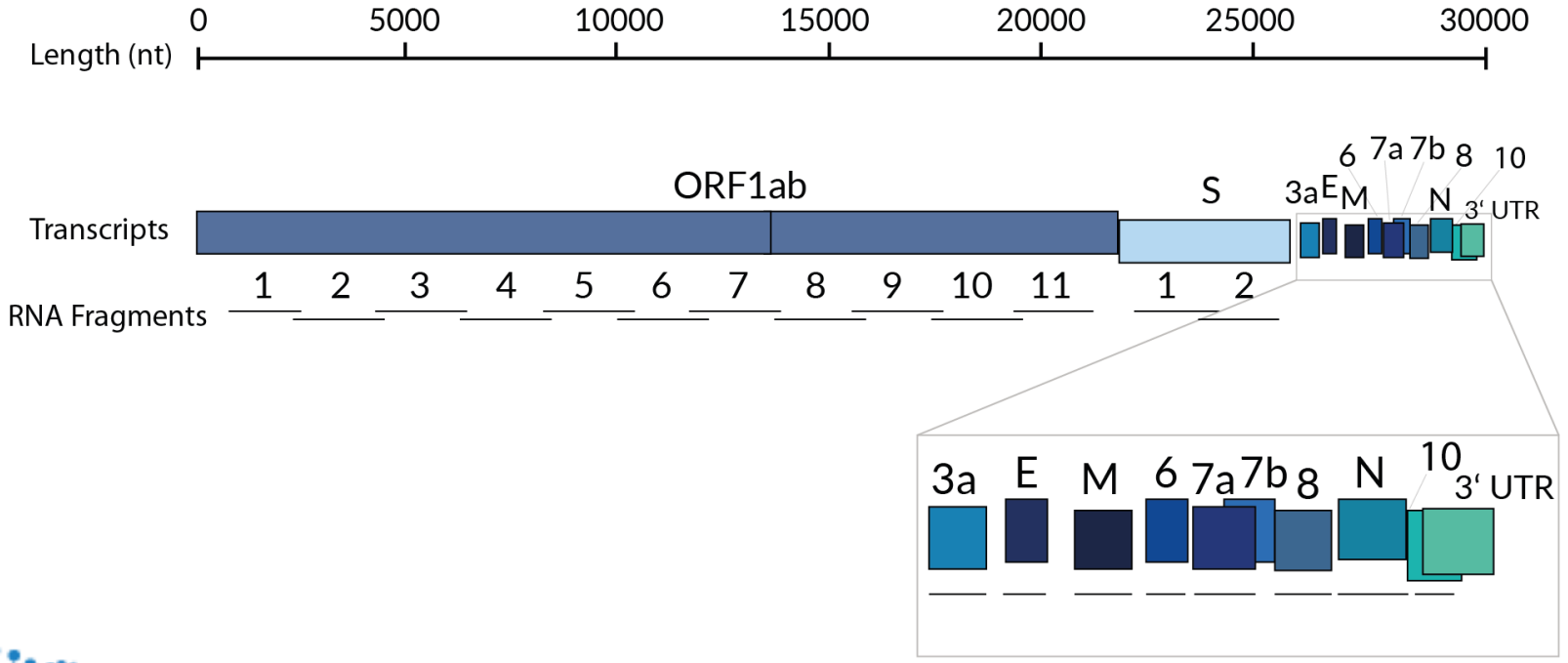
positive control

- SARS-CoV-2 transcriptome in RNA fragments
- Develop and validate Covid19 detection assays

SARS-CoV-2 positive control
For rt-qPCR & LAMP assays

SARS-CoV-2 Positive Control – Features

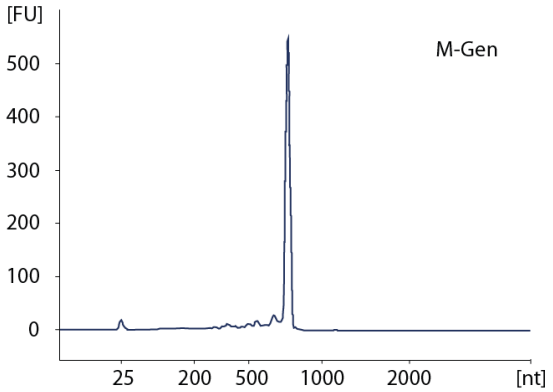
- 98% coverage of transcriptome in RNA Fragments
- 120 – 2000 nts non-infectious RNA Fragments



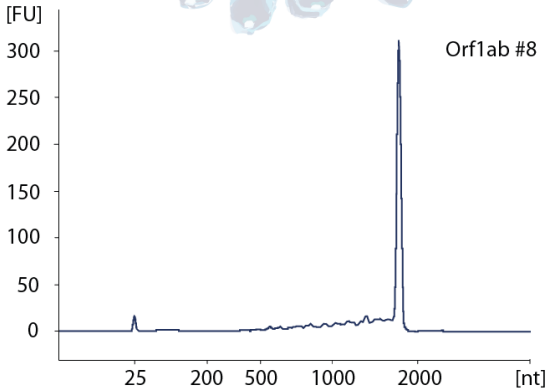
SARS-CoV-2 Positive Control – Quality verified

Fragment	Length (nt)
ORF1ab #1 - #11	1800-2000
S #1, #2	2000
ORF3a	830
E	230
M	670

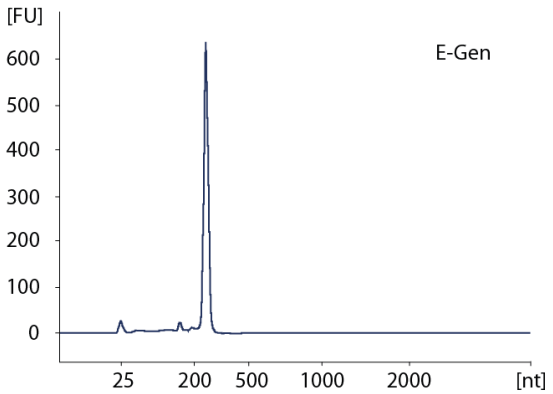
Fragment	Length (nt)
ORF6	190
ORF7a	370
ORF8	370
N	1260
ORF10	120



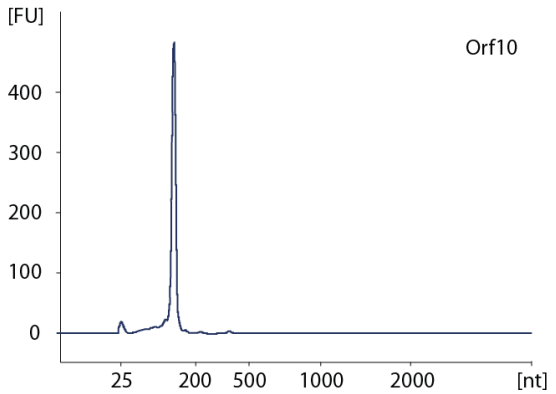
Bioanalyzer analysis of M-Gen with a fragment length of 670 nt.



Bioanalyzer analysis of Orf1ab Fragment #8 with a fragment length of 1800 nt.



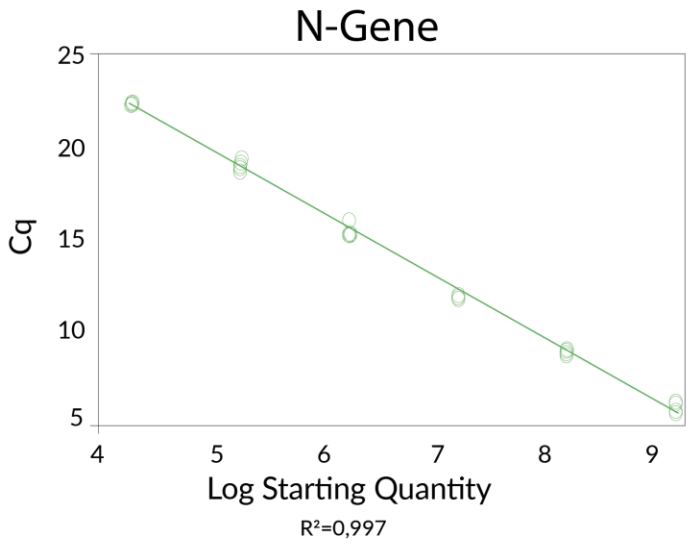
Bioanalyzer analysis of E-Gen with a fragment length of 230 nt.

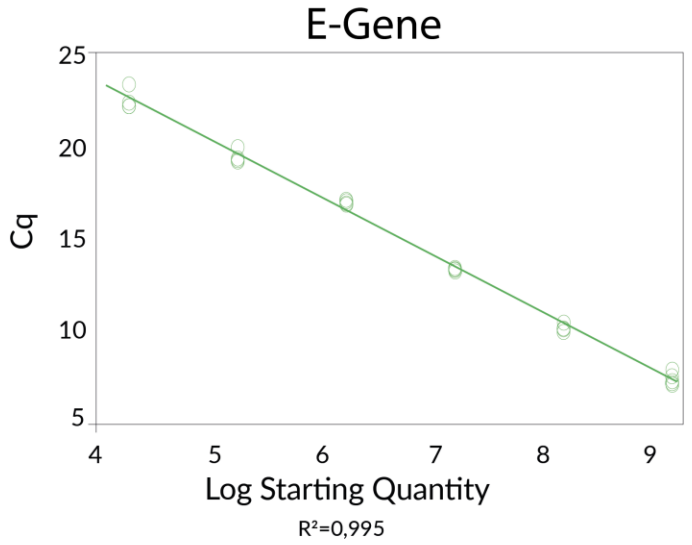


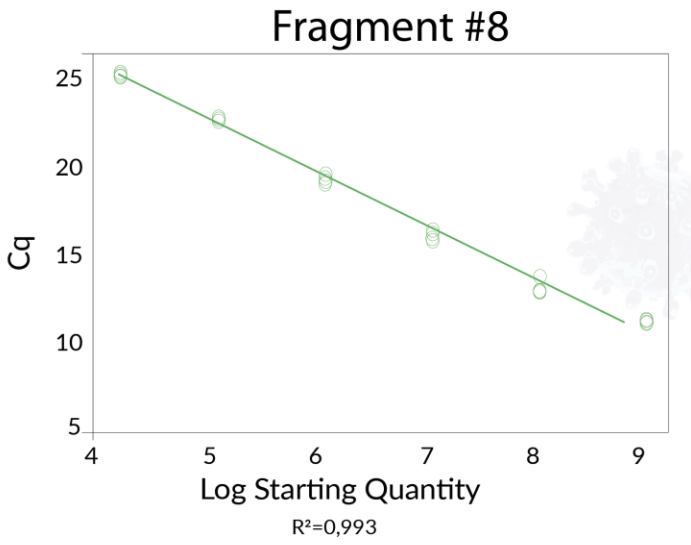
Bioanalyzer analysis of Orf10 with a fragment length of 120 nt.

SARS-CoV-2 Positive Control – Wide Detection Range

- Real-time quantitative PCR
  - primers against fragments ORF1ab #8, E and N
  - good detection sensitivity across six log-dilutions







Suggested input amount: 100 pg of SARS-CoV-2 positive control

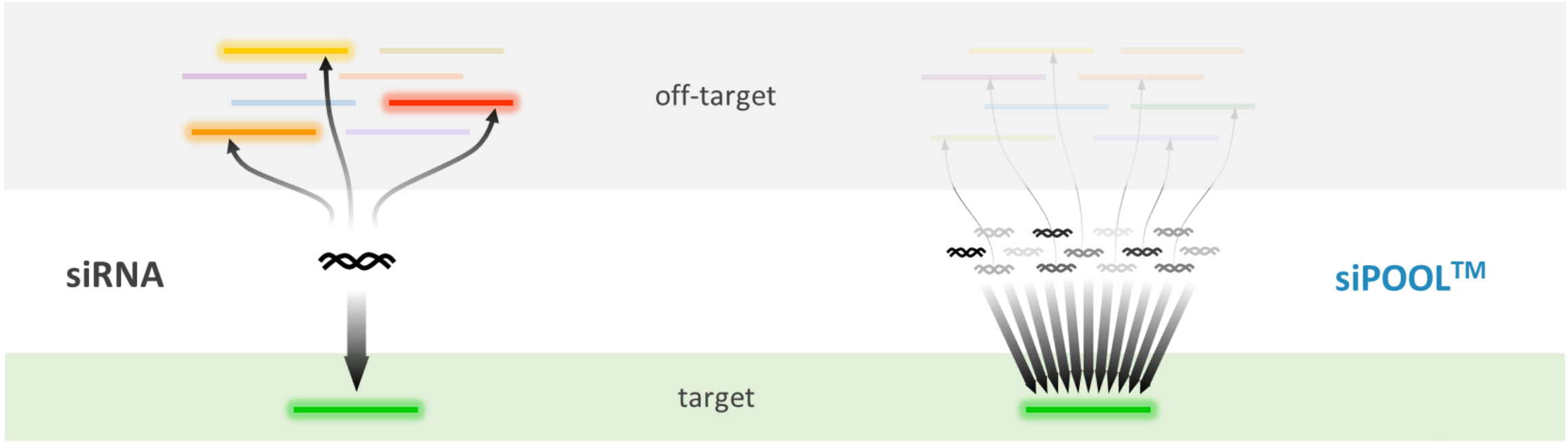
Summary – siTOOLs Biotech SARS-CoV-2 positive control

- 98 % coverage of SARS-CoV-2 transcriptome in RNA fragments
- Wide detection range
- Quality verified
- Available in large Scales (in 20 and 100 reactions sizes)

siPOOLs

For Rapid & Reliable Gene Silencing

The Power of Complex POOLing



- Multiple off-targets
- Low or variable efficiency

- High target specificity
- Increased efficiency & reproducibility

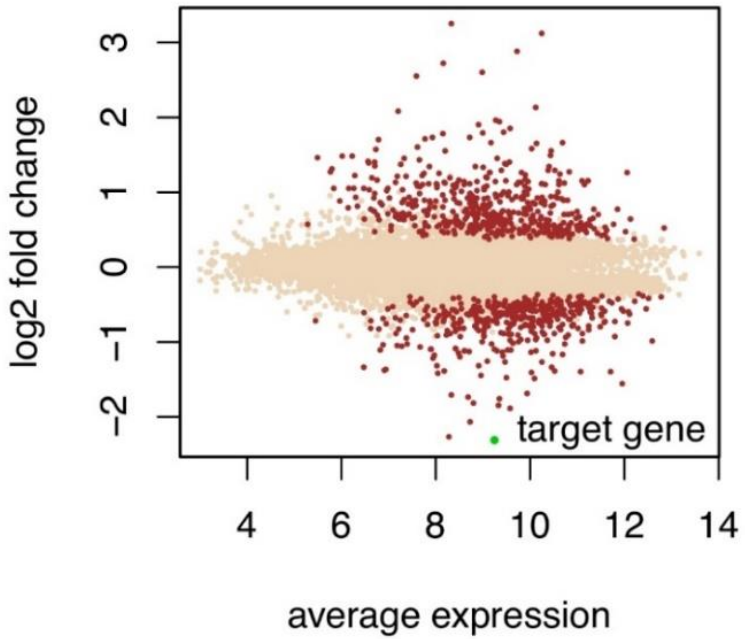
⇒ **RNA interference (siPOOL™)**

⇒ **RNA affinity purification (raPOOL™)**

⇒ **Ribosomal RNA depletion (riboPOOL™)**

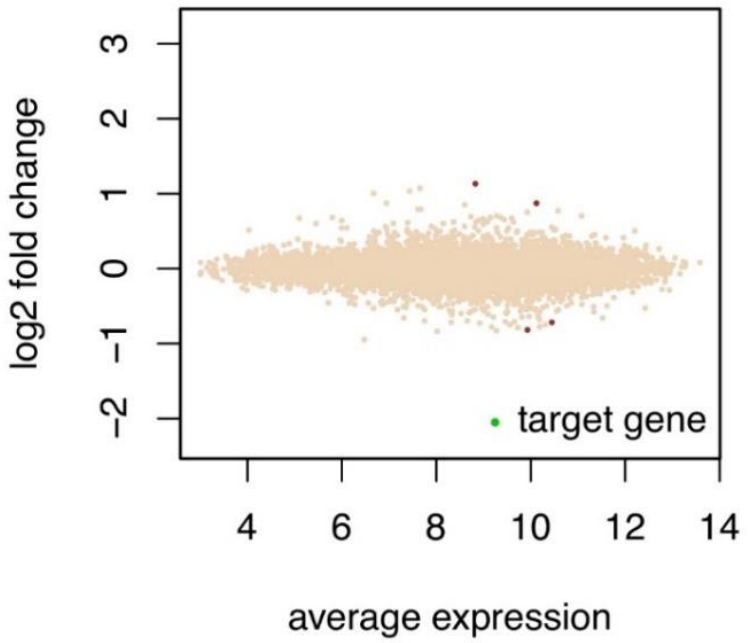
siPOOLs counter Off-target Effect in RNAi

Single siRNA



siRNA within siPOOL

siPOOL



- *HeLa cells*
- *3 nM Scyl1 siRNA or siPOOL*
- *48 h*
- *whole transcriptome profiling by Affymetrix Microarray*

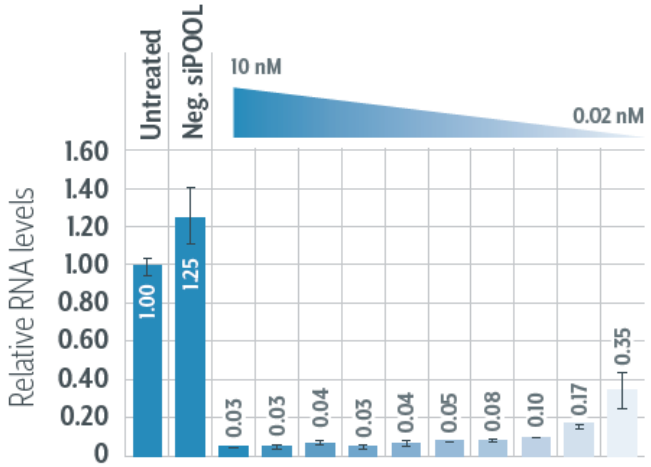
POOLing reduces off-target effects

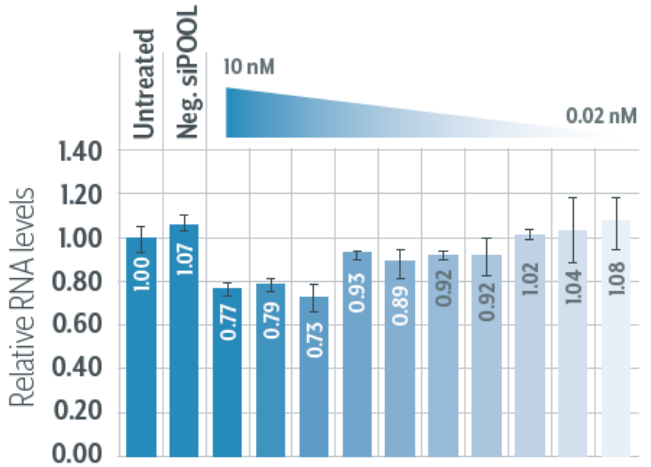
Hannus et al., Nucleic Acids Res, 2014

Increased Efficiency with siPOOL for Gene Silencing

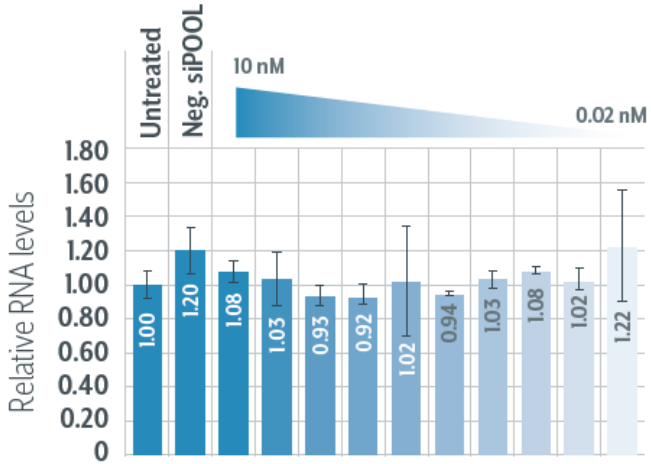
siTOOLs BIOTECH

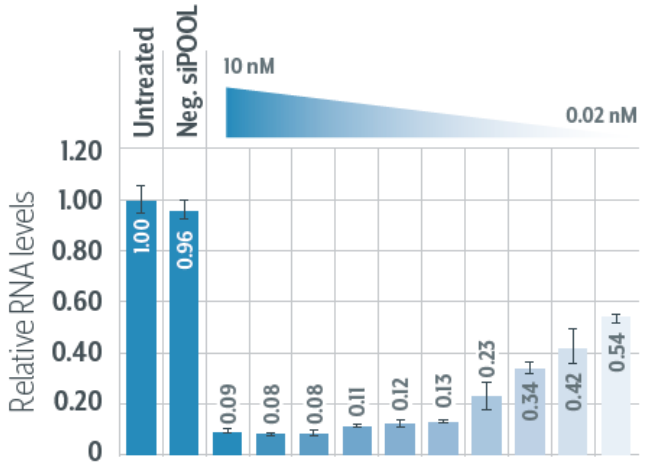
RT-PCR
SMARCA2





RT-PCR
SMARCA4



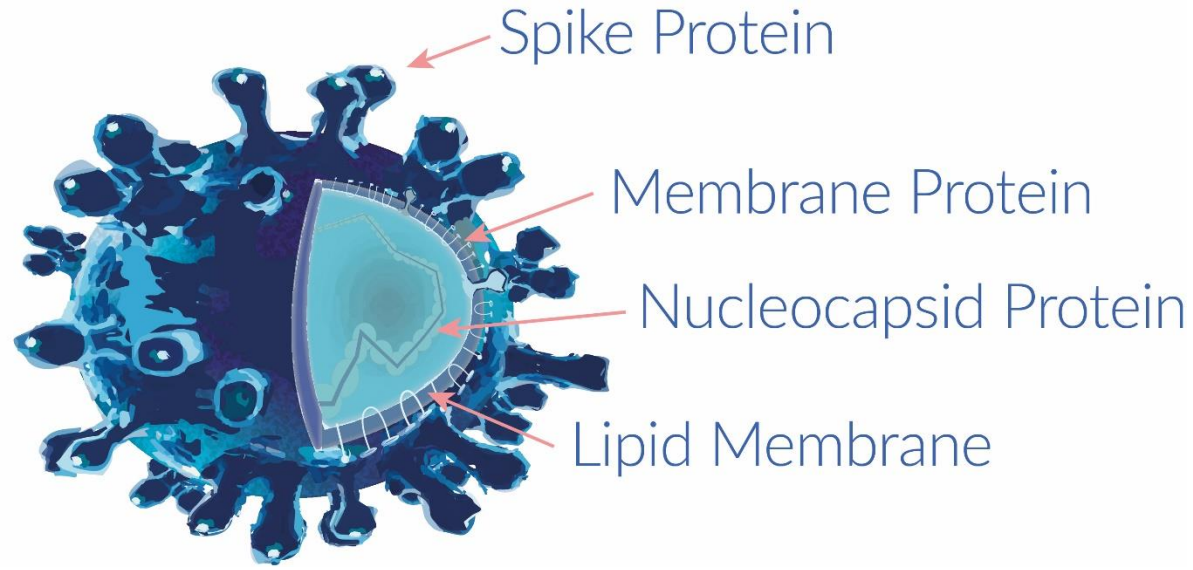


Dr. Mona Malz, PhD
Senior Scientist
Cancer Drug Discovery
German Cancer Research Center (DKFZ)
Heidelberg, Germany

dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

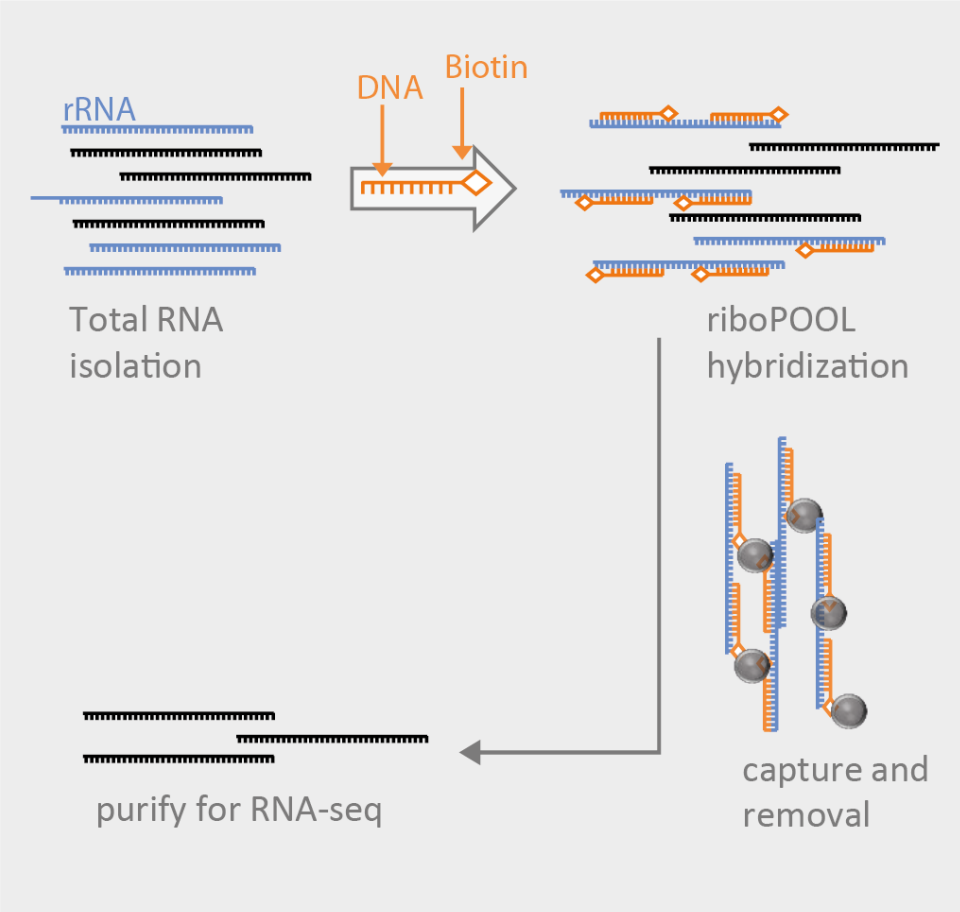
siPOOLs - SARS-CoV-2 Research

- RNAi Knockdown of SARS-CoV-2 transcripts
- RNAi Knockdown of human host factors
  - ACE2
  - TMPRSS2
- siPOOLs in combination



riboPOOLs
for efficient & robust ribosomal RNA depletion

riboPOOLs – Hybridization-based rRNA Depletion



25 min **Hybridization**

riboPOOLs are resuspended and hybridized to DNA-free total RNA (input range: 100ng -5µg).

30 min **Capture & Removal**

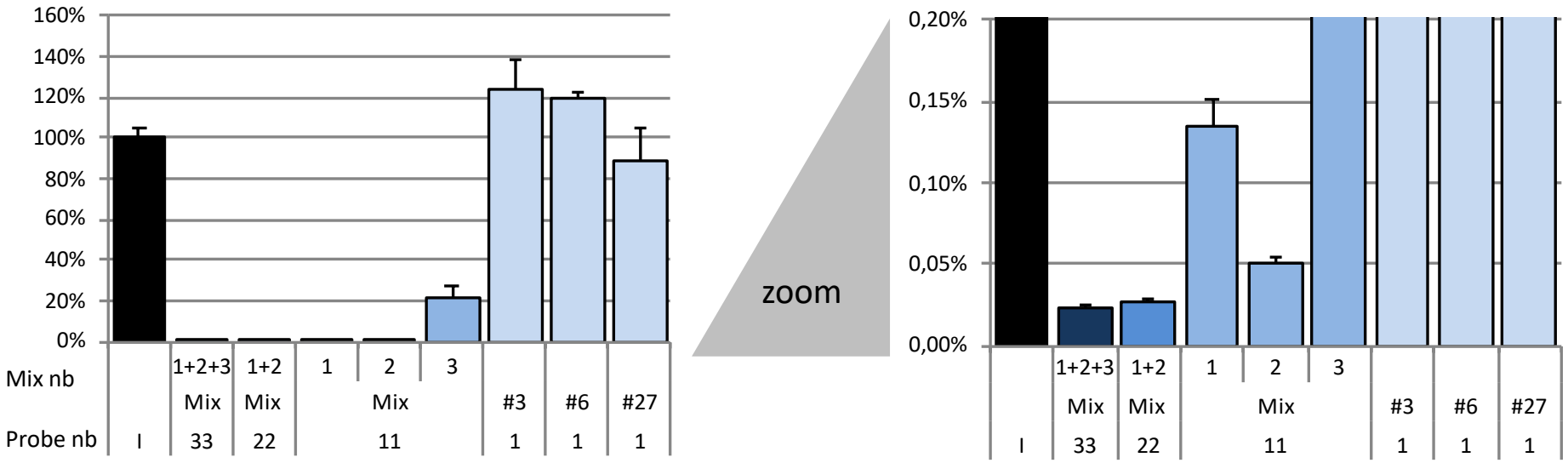
Streptavidin-coated magnetic beads separate riboPOOL-bound rRNAs.

15-90 min* **Purification**

Remaining relevant RNAs are purified by ethanol, silica column or SPRI beads prior to downstream analysis.

**Time required dependant on clean-up method*

Ribosomal RNA Depletion – Complexity Matters



⇒ **depletion efficiency increases with pool complexity**

Diverse riboPOOLs for Single/ Multi-Species RNA-Seq

siTOOLs BIOTECH

single species

Eukaryotes

Homo sapien (human)
Mus musculus/Rattus norvegicus (mouse/rat)
Drosophila melanogaster (Fly)
Arabidopsis thaliana
Danio rerio (Zebrafish)
Saccharomyces cerevisiae
Pichia pastoris
Bombyx morii
Ustilago maydis
Amphimedon queenslandica
Schmidtea mediterranea
Plautia stali
Chinchilla Laginera
Oryza sativa
Loripes + Lucinoma
Ixodes scapulars (Ticks)
Aedes albopictus (Mosquito)

Prokaryotes

Escherichia coli
Bacillus subtilis
Caulobacter crescentus
Clostridium perfringens
Pseudomonas aeruginosa
Staphylococcus aureus
Salmonella enterica
Stenotrophomonas sp
Haloferax volcanii

multiple species

Pan-riboPOOLs

Pan-riboPOOLs*
Pan-riboPOOLs are used for versatile single-step rRNA depletion across closely-related groups of species.

Eukaryotes	Prokaryotes
Pan-Mammals	Pan-Prokaryotes
Pan-Fungi	Pan-Bacteria
Pan-Plants	Pan-Archaea
Pan-Birds	

Combination riboPOOLs

For single-step rRNA depletion across diverse species groups.

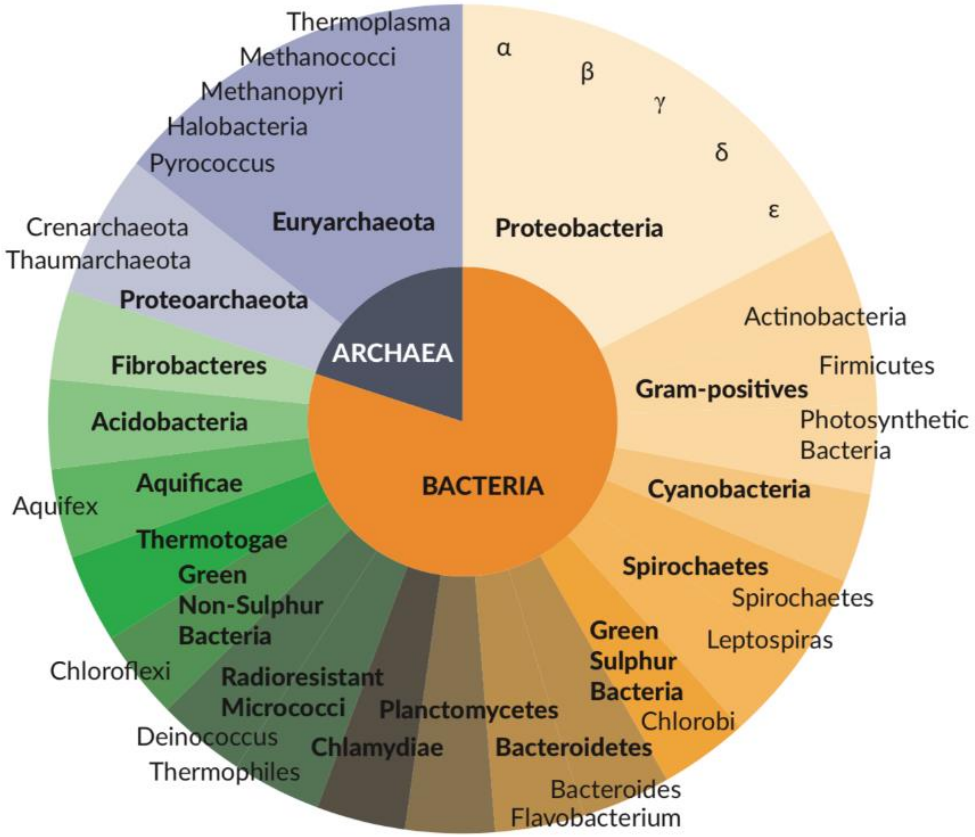
- Up to 4 existing riboPOOLs
- Custom combination ratio (to reflect level of RNA from each species/species group in sample)

other abundant RNA

human Globin mRNA
PolyA

Species not listed? Create a Custom riboPOOL with our One-Time Custom riboPOOL Setup Service

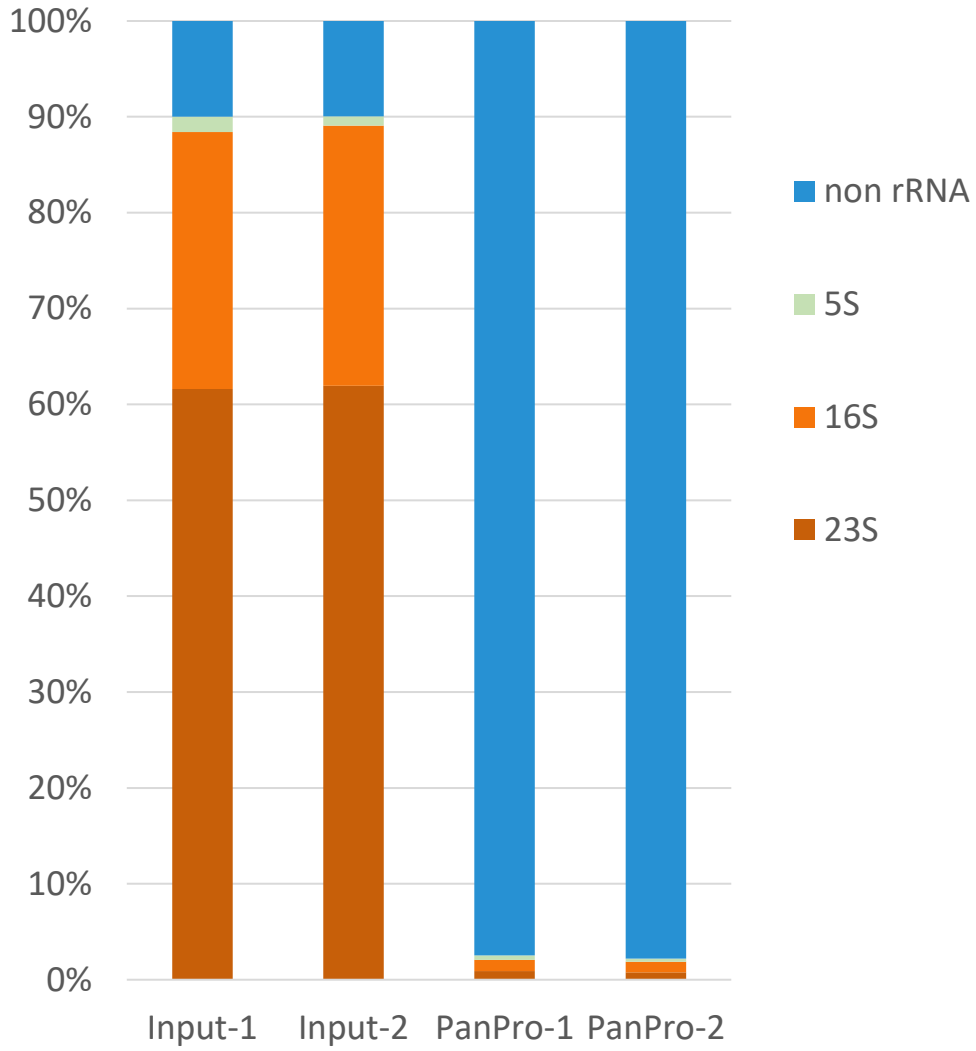
Pan-Prokaryote riboPOOLs – Wide Species Coverage



Efficiencies may vary across sample types and experimental conditions.

Species tested and validated by RNA-Seq
Acetobacterium woodii
Acinetobacter baumannii
Bacteroides thetaiotaomicron
Bifidobacterium breve
Burkholderia glumae
Clostridium saccharoperbutylacetonicum
Escherichia coli
Fusobacterium nucleatum
Hemophilus influenza
Janthinobacterium sp.
Moorella thermoacetica
Pseudomonas aeruginosa
Pseudomonas putida
Salmonella typhimurium
Sinorhizobium fredii
Sporomusa ovata
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Thermoanaerobacter kivui

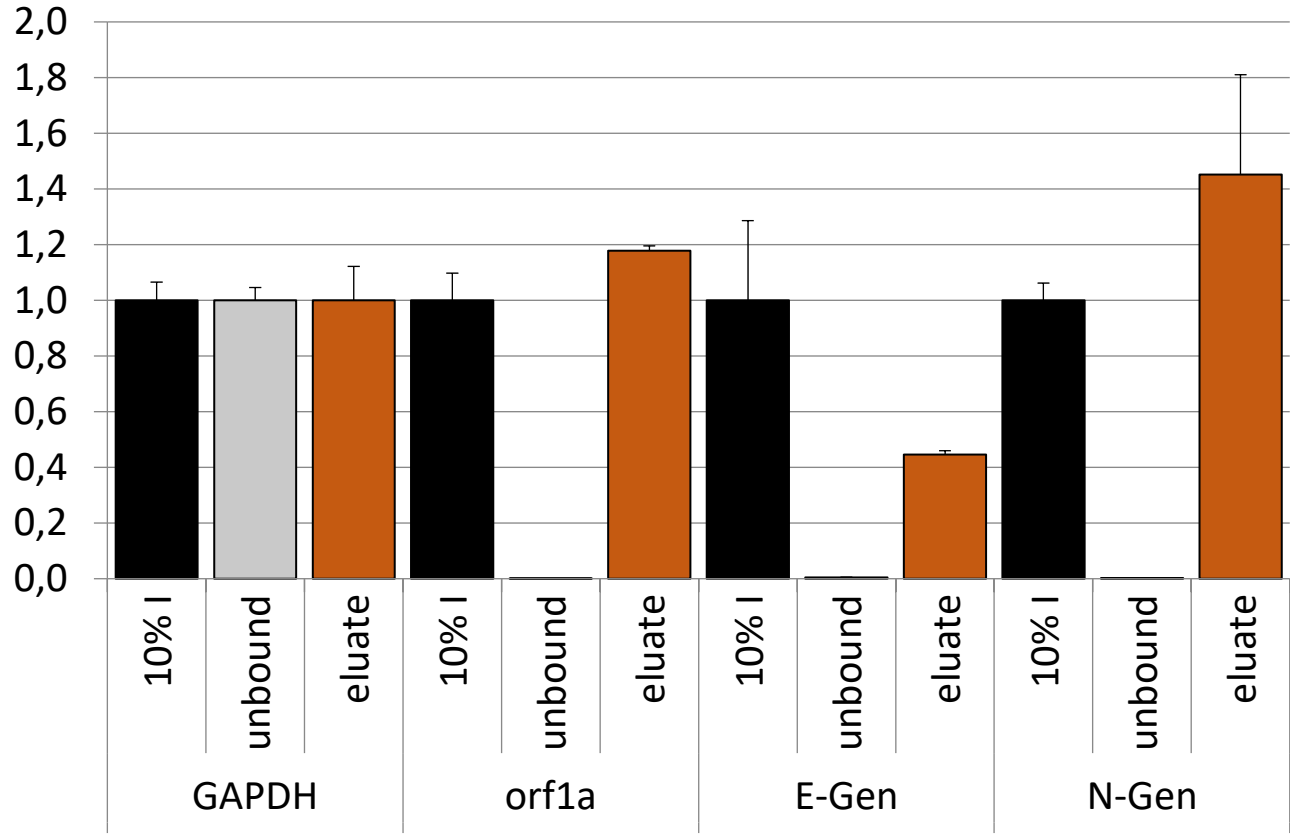
Pan-Prokaryote riboPOOLs – Wide Species Coverage



Experiment Conditions	
Input (ng)	1000 of E. coli RNA
RIN	6,5
RNA Isolation	Nucleo Spin RNA 8
rRNA depletion	Pan-Plant riboPOOL Kit
RNA clean-up	RNA Cleanup XS
Library prep	NEB Kit (modified)
Sequencing	Illumina HiSeq300

riboPOOLs – SARS-CoV-2 Research

Deplete SARS-CoV-2 RNA



Unbound = Supernatant
Eluate = Bound to probes

riboPOOLs – SARS-CoV-2 Applications

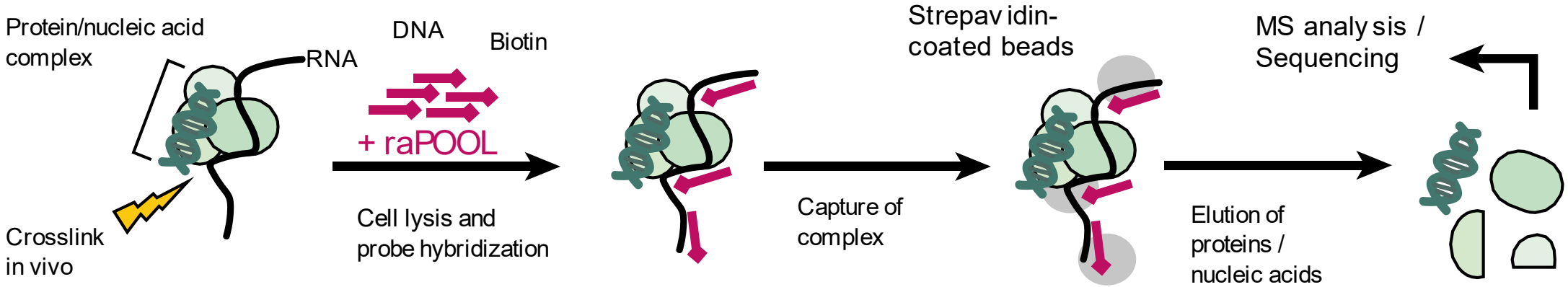
1. Study microbial changes upon SARS-CoV-2 infection
2. Study host gene expression patterns

~99 % depletion efficiency in infected human samples

raPOOLs
For Robust & Targeted RNA Capture

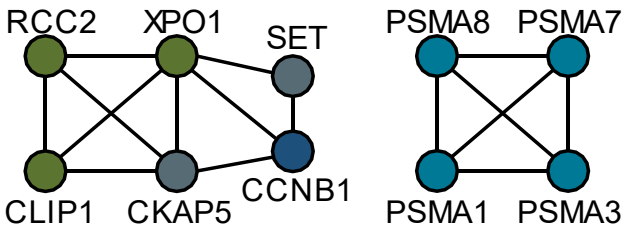
raPOOLs – Targeted RNA Capture for Biochemical Analysis

- highly complex mixture of 30 biotinylated ssDNA probes
  - RNA Enrichment
  - Interactome Studies
- Thorough design
  - Whole transcript is targeted
  - off-target filters



raPOOLs – SARS-CoV-2 RNA Enrichment

- Study SARS-CoV-2 RNA properties
- Identify viral RNA for RNA modifications
- Identify & characterize SARS-CoV-2 RNA interacting partners



raPOOLs enabled Characterization of Interactome of lncRNA LINC00152

Nötzold et al., Scientific Reports, 2017

Summary

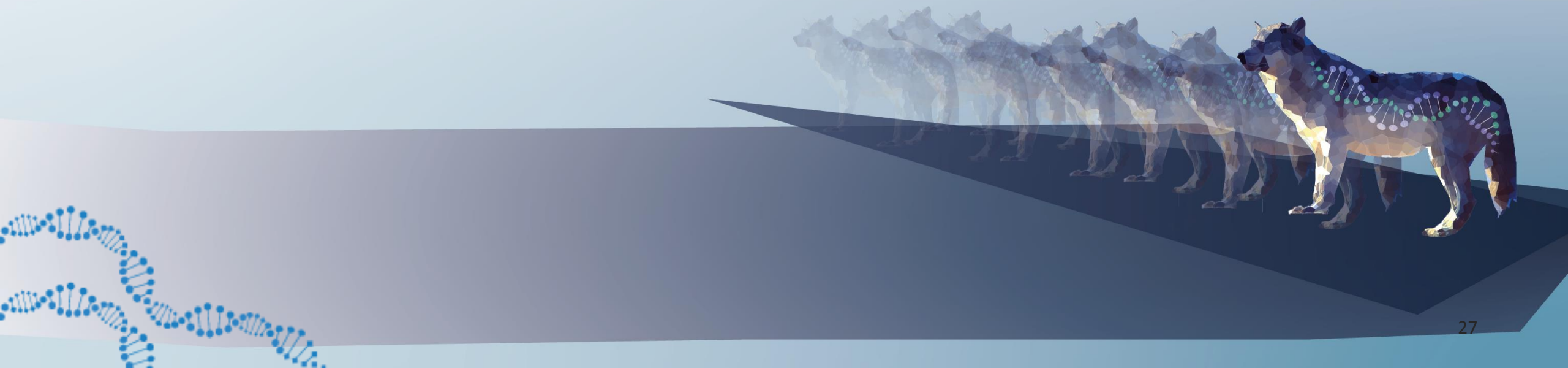
- Pooling oligos (siRNA, biotinylated probes) increase specificity & efficiency of targeting
- SARS-CoV-2 positive control
  - More than 98% transcriptome coverage
  - Wide detection range
- siPOOLs for SARS-CoV-2
  - Greatly reduced off-targeting
  - Highly efficient gene silencing
- riboPOOLs / raPOOLs for SARS-CoV-2
  - Efficient rRNA depletion / targeted RNA Pulldown
  - Multiple Applications – Study Interacting Partners / Host or Microbial Gene Expression Analysis
- Excellent Customer/Technical Support

Thank You For Your Attention, Questions?

Contact us or your local distributor for questions/orders

@siTOOLsBiotech Email: info@sitools.de

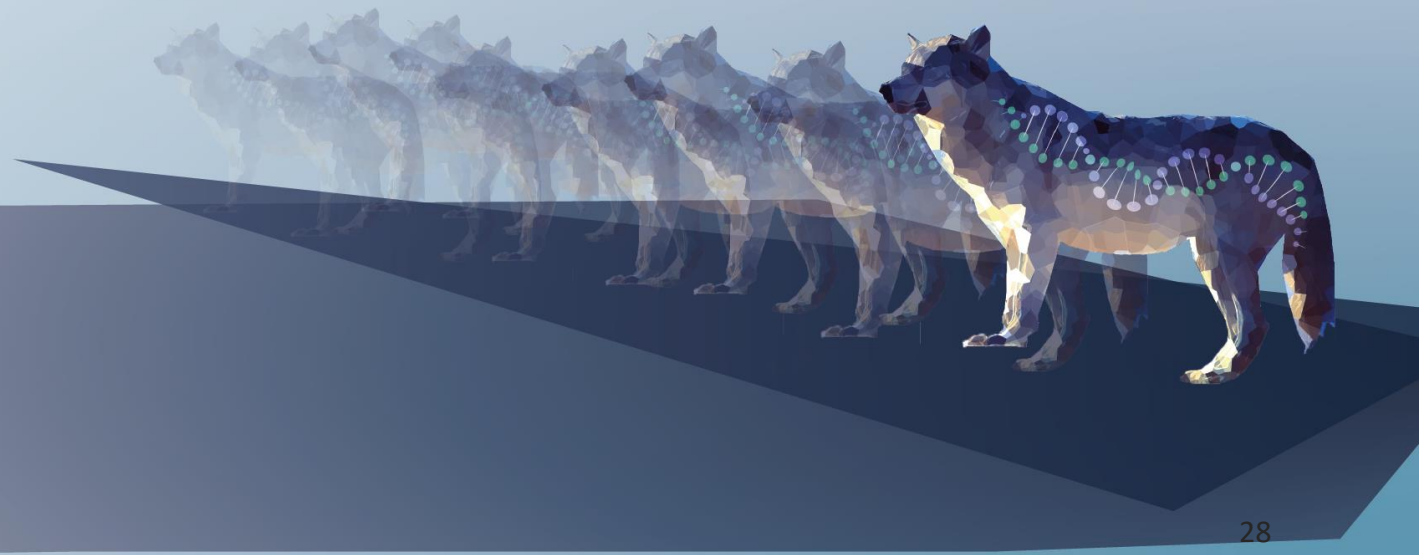
www.sitoolsbiotech.com



siPOOLs - Scales & Formats

- Individual siPOOLs
- siPOOL kits with a standard negative control siPOOLs
- Several positive control siPOOLs
- In tubes or wells/plates

- 5 nmol
- 10 nmol
- 20 nmol
- Bulk orders (10 or more) 2 nmol
- Bulk orders (100 or more) 1 nmol



riboPOOLs - Scale & Formats

riboPOOL Kit

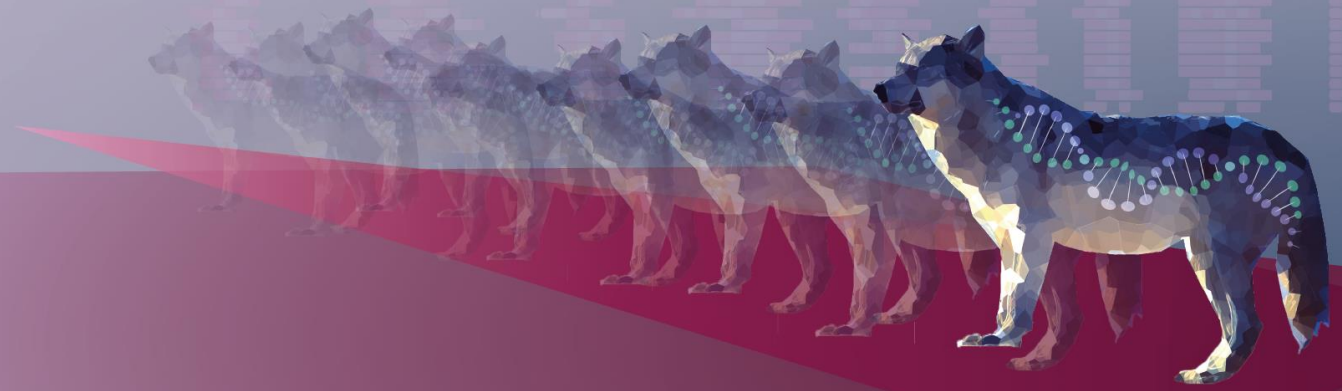
- All reagents up to library preparation included
- Manual provided
- Reaction sizes: 12, 24, 96
  A 6 reaction trial kit is available for first-time purchase, please contact us for a quote.

riboPOOL probe

- riboPOOL probes plus nuclease-free water provided
- Protocol on our website
- Reaction sizes: 20 (2 nmol), 50 (5 nmol), 100 (10 nmol)

raPOOLs – Scales & Format

- raPOOL probes plus nuclease-free water provided
- Protocol on our website
- Reaction sizes: 20 (2 nmol), 50 (5 nmol), 100 (10 nmol)

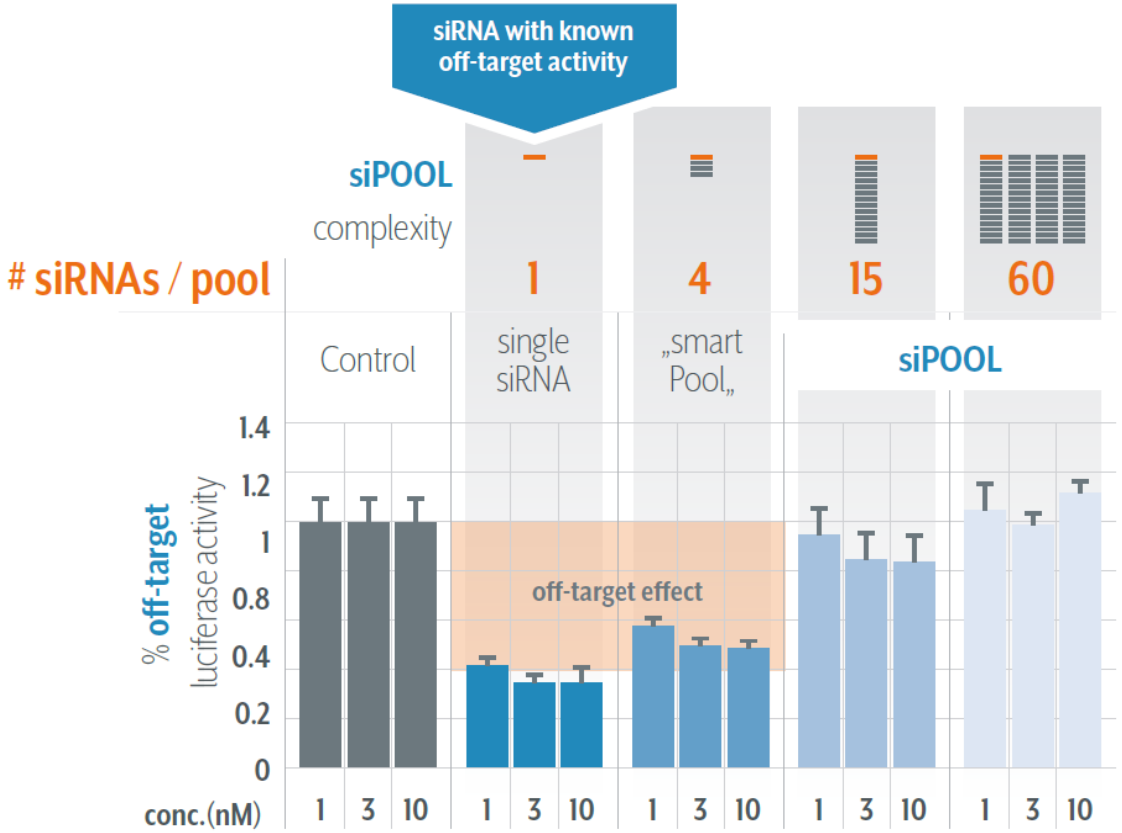


siTOOLs Biotech SARS-CoV-2 positive control - Scales

Product	Catalogue No.	Reactions	Scale (ng)
SARS-CoV-2 positive control 20	cv-C020-000567	20	2
SARS-CoV-2 positive control 100	cv-C100-000567	100	10

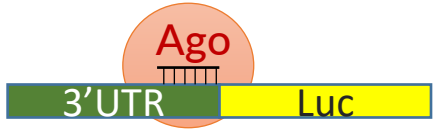
Why 30? Because 4 is not enough!

Off-target spiking experiment



Hannus et al., Nucleic Acids Res, 2014

Off-target luciferase reporter



- *HeLa cells*
- *10 nM Scyl1 siRNA/siPOOL*
- *24-48 h*
- *reporter assay, RT-PCR, Western, functional assay*

High siRNA complexity (> 15 siRNAs) needed to robustly reduce off-target effects