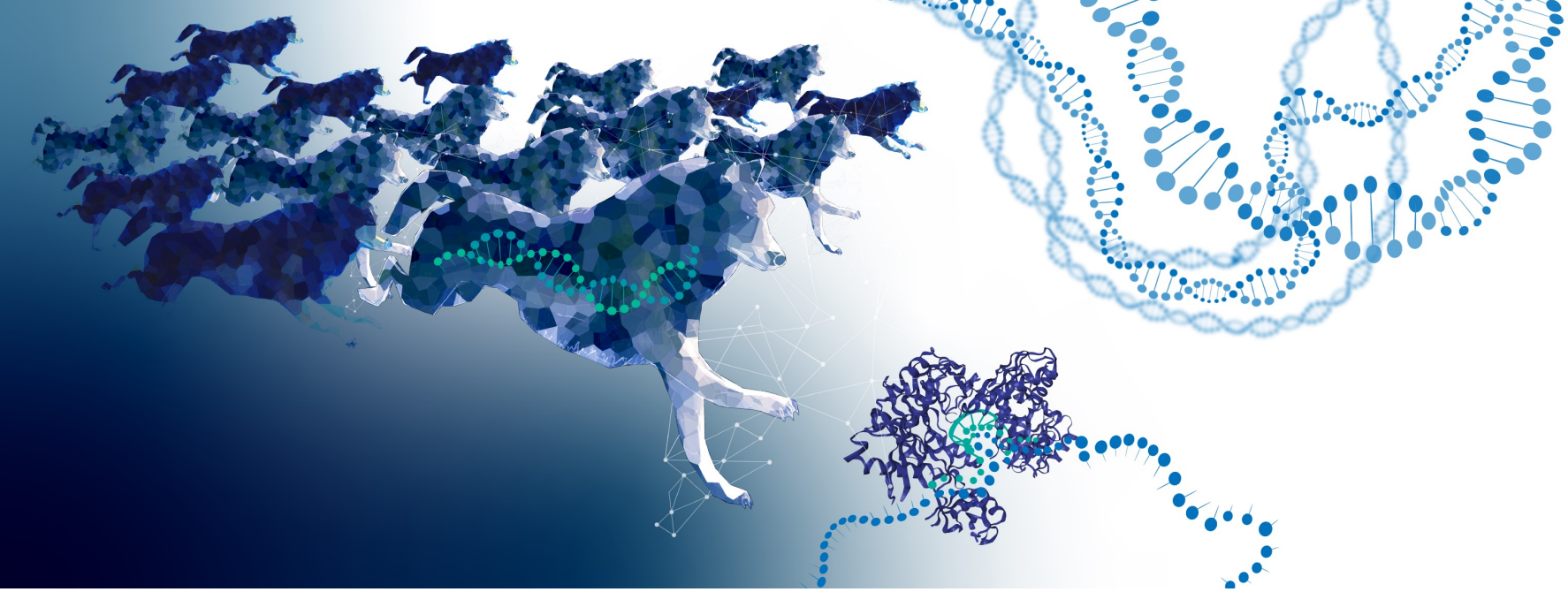


siTOOLs Biotech Introduction

Products and Services

Speaker: Anna Liznar

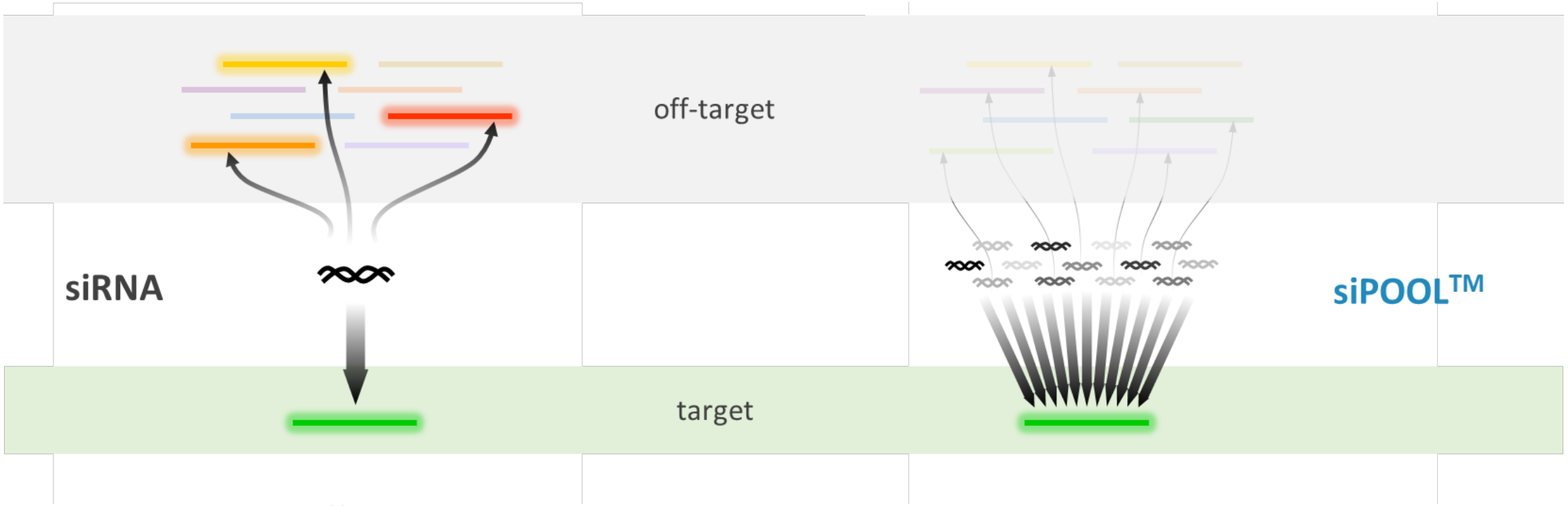
siTOOLs BIOTECH

www.sitoolsbiotech.de

Products & Services

- **Our Products:** highly **complex**, optimally designed oligonucleotide **pools**
  - siPOOLs
  - riboPOOLs
  - raPOOLs
- **Our Services:**
  - Big data analysis (RNAi & CRISPR)
  - RNAi screening/expression analysis projects
  - NGS services

Power of Pooling



- Multiple off-targets
- Low or variable efficiency

- High target specificity
- Increased efficiency & reproducibility

⇒ **RNA interference (siPOOL™)**

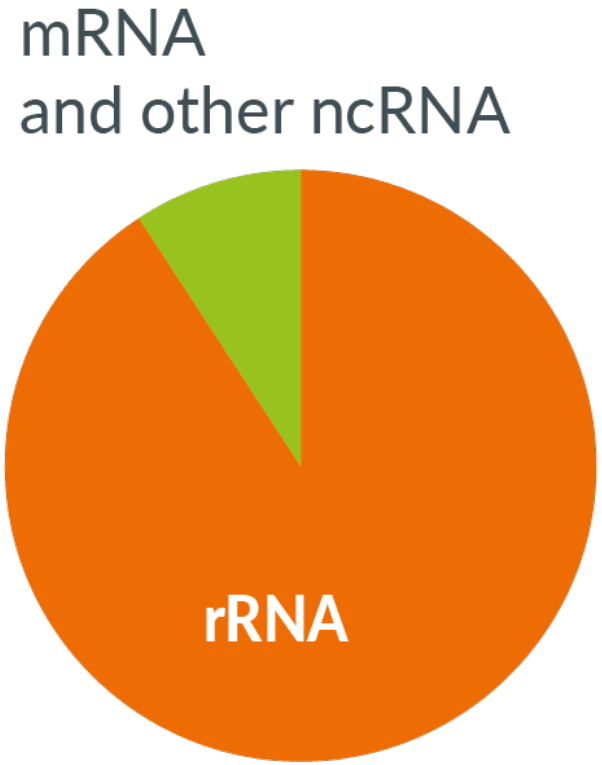
⇒ **RNA affinity purification (raPOOL™)**

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

riboPOOLs

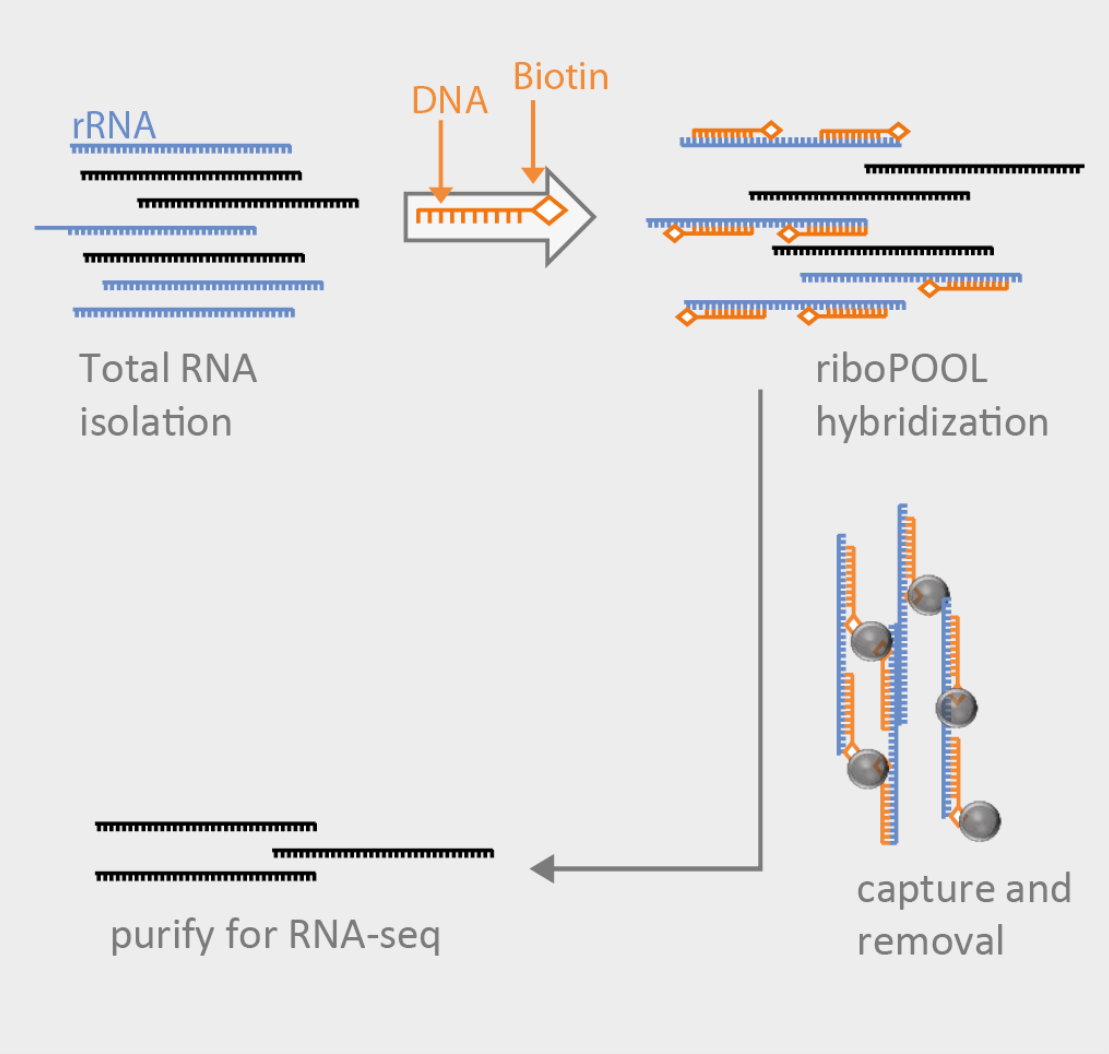
for efficient & robust ribosomal RNA depletion

Why do rRNA depletion?



- Ribosomal RNAs (rRNA): 80-90% of total RNA for most species
- Limits detection of relevant RNAs – messenger RNA (mRNA) and non-coding RNA (ncRNA)
- Cost savings for RNA-Seq

rRNA depletion with riboPOOLs - Workflow



25 min **Hybridization**

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

30 min **Capture & Removal**

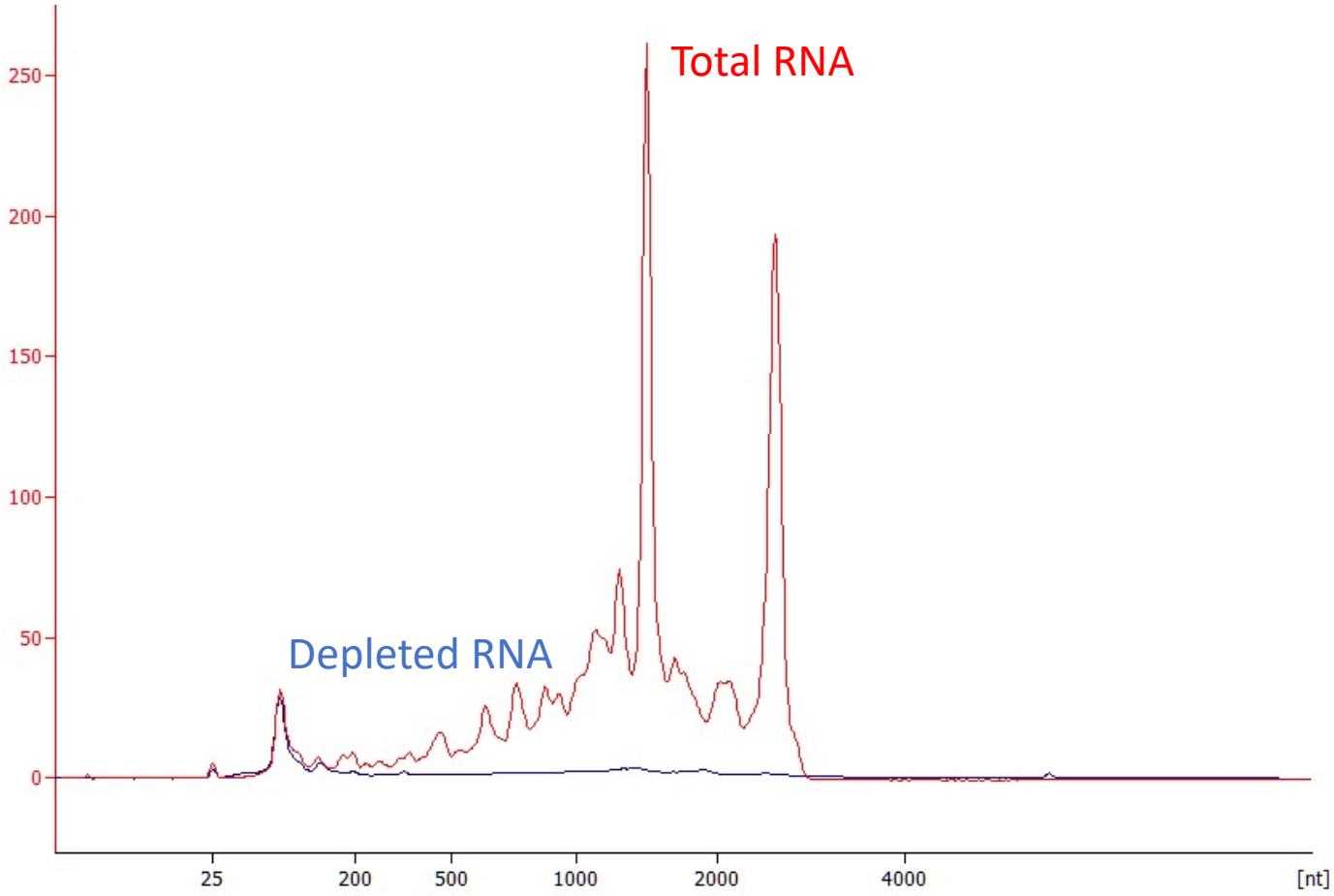
Streptavidin-coated magnetic beads separate riboPOOL-bound rRNAs.

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*

riboPOOLs for Any Species or Abundant RNA



Pan-Archaea riboPOOL

riboPOOLs for Any Species or Abundant RNA

single species riboPOOLs

Eukaryotes

Aedes albopictus riboPOOL
Amphimedon queenslandica
Arabidopsis thaliana riboPOOL
B. mori riboPOOL
Chinchilla lanigera riboPOOL
Crassostrea gigas riboPOOL
Chlamydomonas rheinhardii
Danio rerio riboPOOL
D. melanogaster riboPOOL
Emiliania huxleyi riboPOOL (Algea)
Gallus gallus domesticus riboPOOL
human riboPOOL
human/mouse/rat riboPOOL
Ixodes scapular riboPOOL
Loripes orbiculatus & Lucinoma aequizonata (Clamps)
mouse/rat riboPOOL
Oryza sativa riboPOOL
Pichia pastoris riboPOOL
Plautia stali riboPOOL (bug)
Saccharomyces cerevisiae riboPOOL
Schizosaccharomyces pombe riboPOOL
Schmidtea mediterranea riboPOOL
Staphylococcus aureus riboPOOL
Ustilago maydis riboPOOL

Prokaryotes

Escherichia coli riboPOOL
Bacillus subtilis riboPOOL
Caulobacter crescentus riboPOOL
Clostridium perfringens riboPOOL
Haloferax volcanii riboPOOL
Pseudomonas aeruginosa riboPOOL
Salmonella enterica riboPOOL
Stenotrophomonas sp. riboPOOL
Mycobacterium smegmatis

riboPOOLs for Any Species or Abundant RNA

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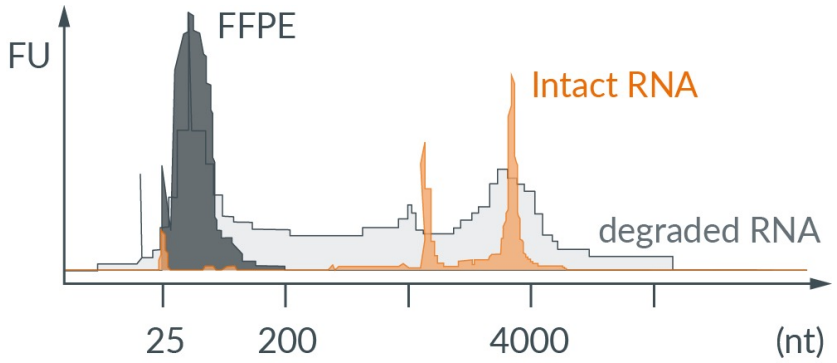
degraded RNA

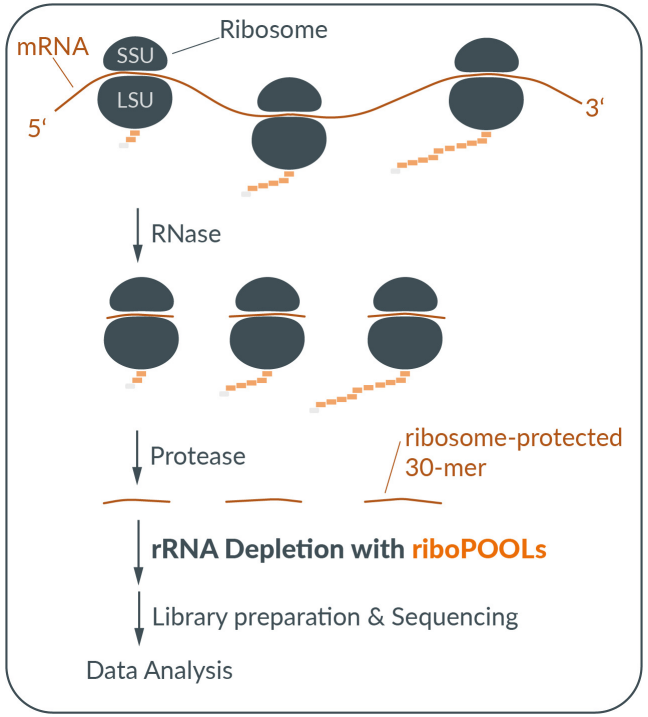
D. melanogaster degraded RNA riboPOOL
human FFPE/degraded RNA riboPOOL
human/mouse/rat FFPE/degraded RNA riboPOOL
mouse/rat FFPE/degraded RNA riboPOOL



FFPE = formalin-fixed or formaldehyde-fixed paraffin-embedded tissue

ribosome Profiling

human Ribo-Seq riboPOOL
human/mouse/rat Ribo-Seq riboPOOL
mouse/rat Ribo-Seq riboPOOL
C. elegans Ribo-Seq riboPOOL

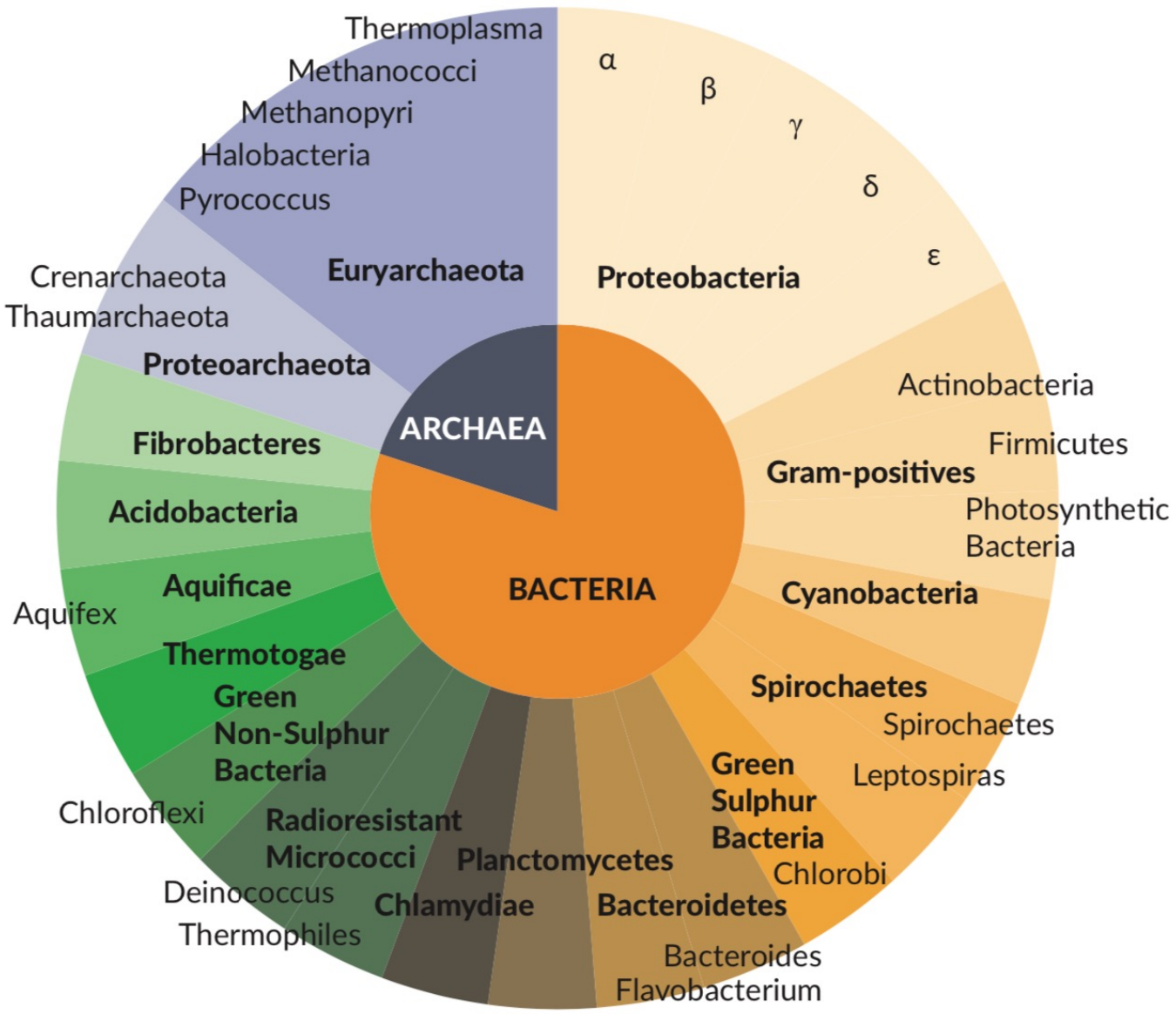


other abundant RNAs

Human globin mRNA
Pola-A tailed RNAs (euk. mRNAs)
SARS-CoV-2 RNA

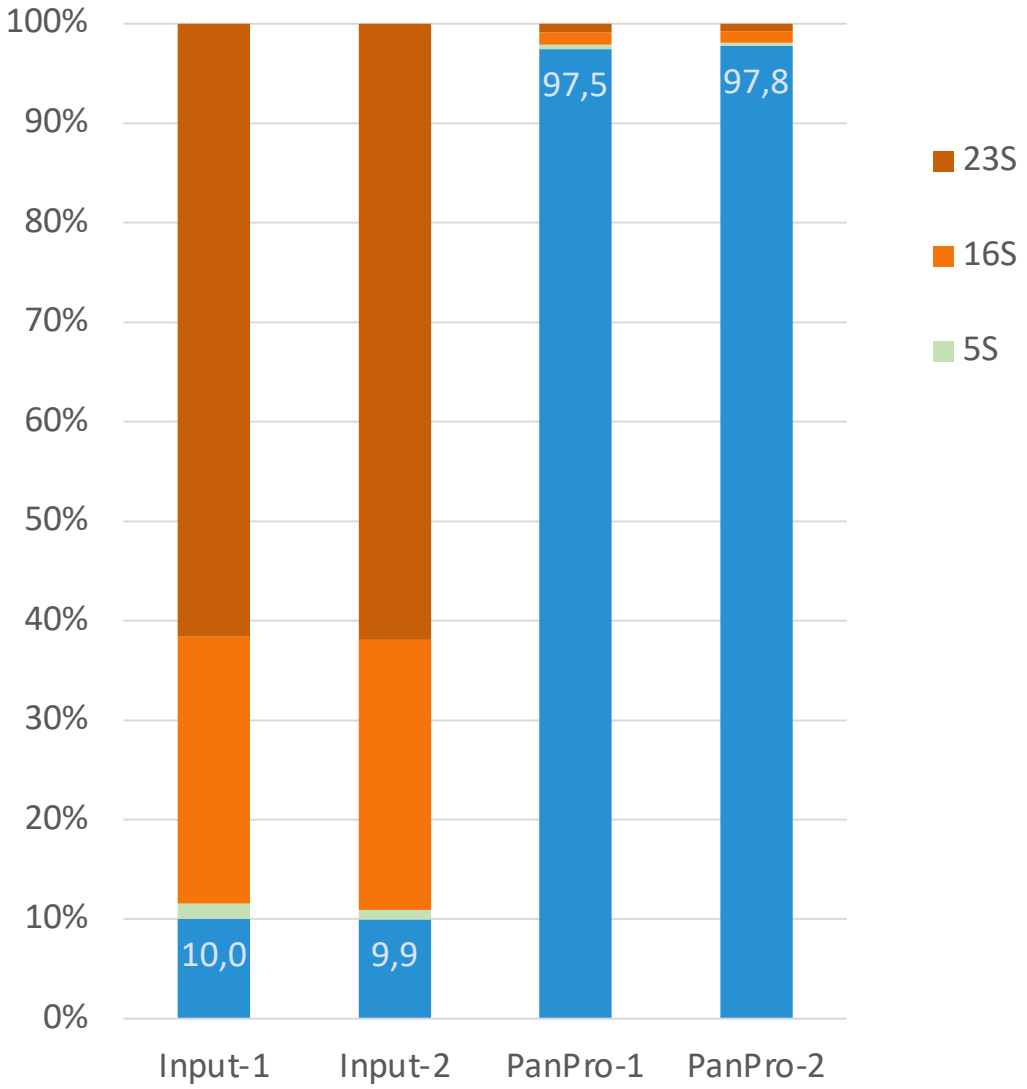
Species not listed?
Create Custom riboPOOL with One-Time riboPOOL Setup Service

Pan-Prokaryote riboPOOL for Universal Microbial rRNA Depletion



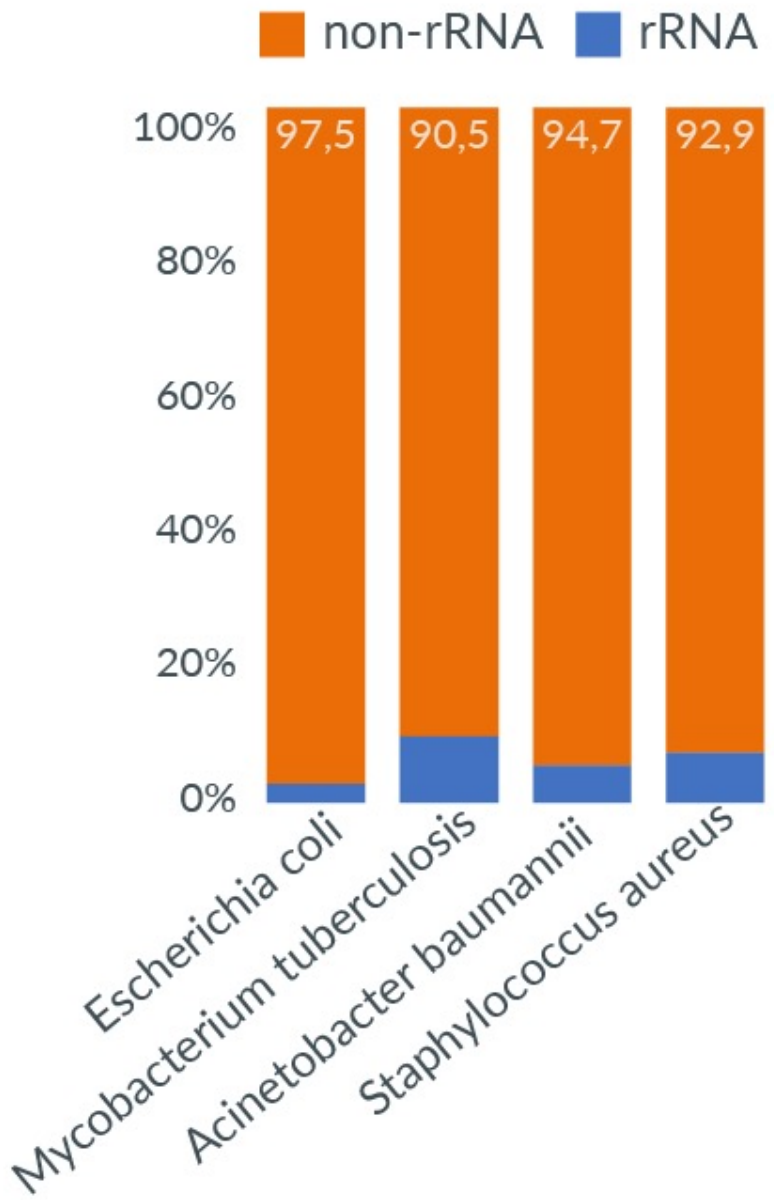
- Broad microbial coverage, suited for Metagenomic/Microbiome analysis
- Highly efficient rRNA depletion
- Targets 5S, 16S and 23S rRNA
- Versatile solution

Pan-Prokaryote riboPOOL for Universal Microbial rRNA Depletion



Experiment Conditions	
Input (ng)	1000 of E. coli RNA
RIN	**~8**
RNA Isolation	QIAgen Rneasy
rRNA depletion	Pan-Pro riboPOOL Kit
RNA clean-up	RNA XPClean (Beckman Coulter)
Library prep	NEB Kit (modified)
Sequencing	Illumina HiSeq300

Pan-Prokaryote riboPOOL for Universal Microbial rRNA Depletion

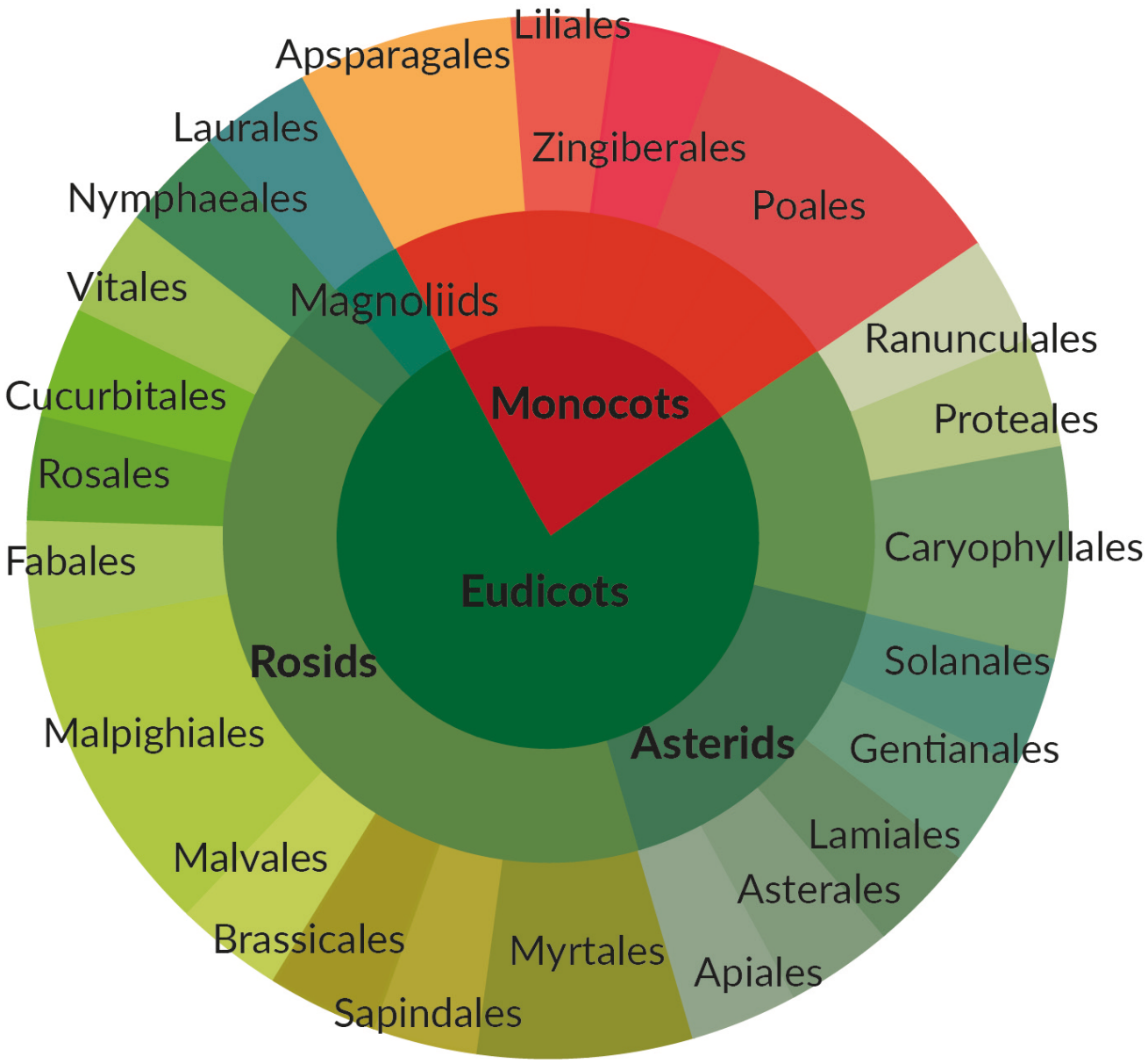


RIN
9,2 - 10
RNA Isolation
Zymo clean and concentrator kit
rRNA depletion
Pan-Prokayrote riboPOOL
Library prep
Illumina Truseq Stranded mRNA Library Prep kit
Sequencing
Illumina NextSeq

Brooke Tomlinson

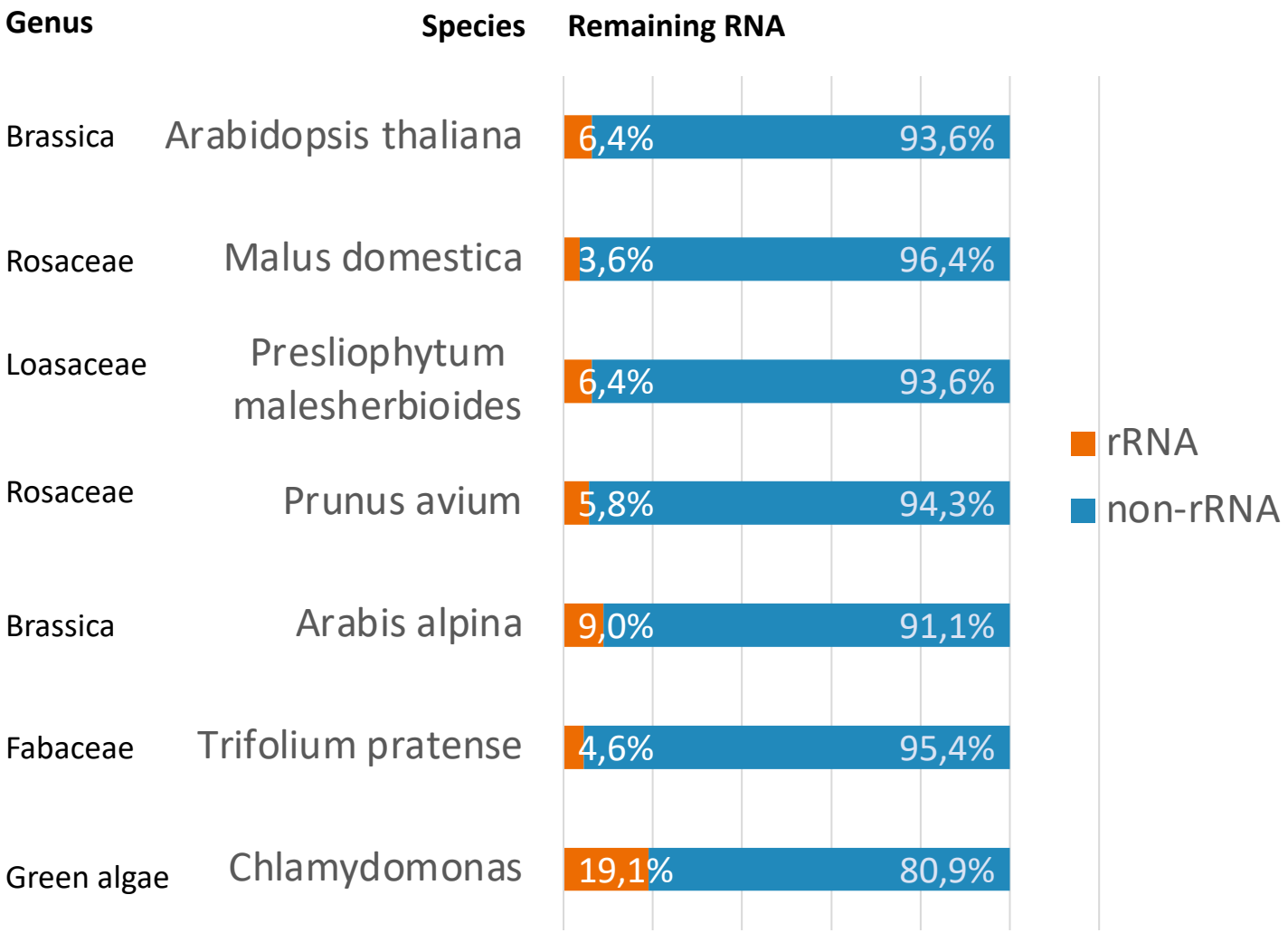
PhD Candidate
University of South Florida
Cell, Molecular, and Microbiology Dept.

Pan-Plant riboPOOL for Universal Plant rRNA Depletion



- Efficient rRNA depletion tool
- Broad coverage of flowering plants
- For leaf, seed & root tissue
- Targets 28S, 18S, 5.8S & 5S rRNA
- Targets mitochondrial rRNA
- Targets plastid rRNA

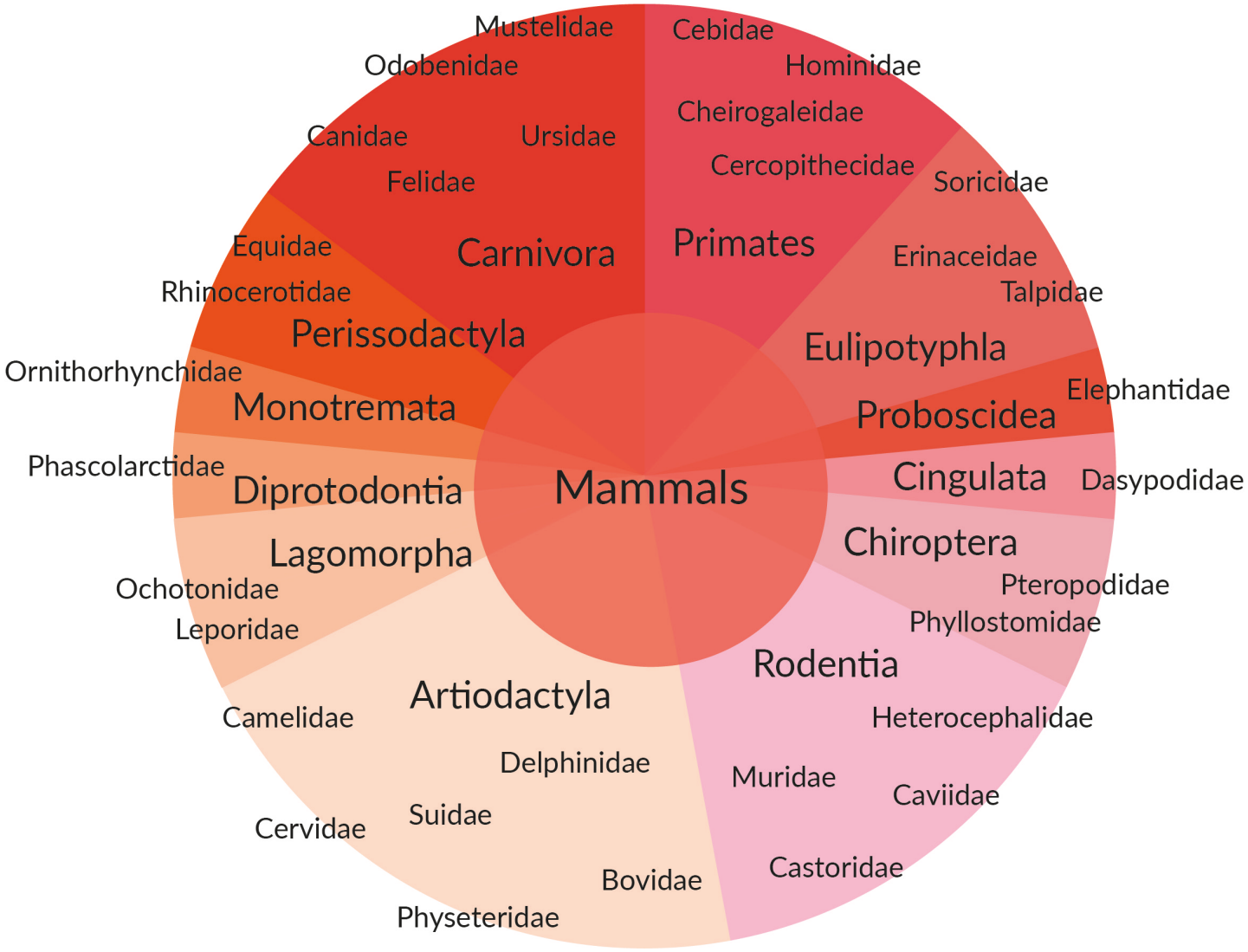
Pan-Plant riboPOOL Efficient Across Genera



Dr. Bruno Huettel and Dr. Christian Wöhle,
Max Planck Genome Centre Cologne (MPI)

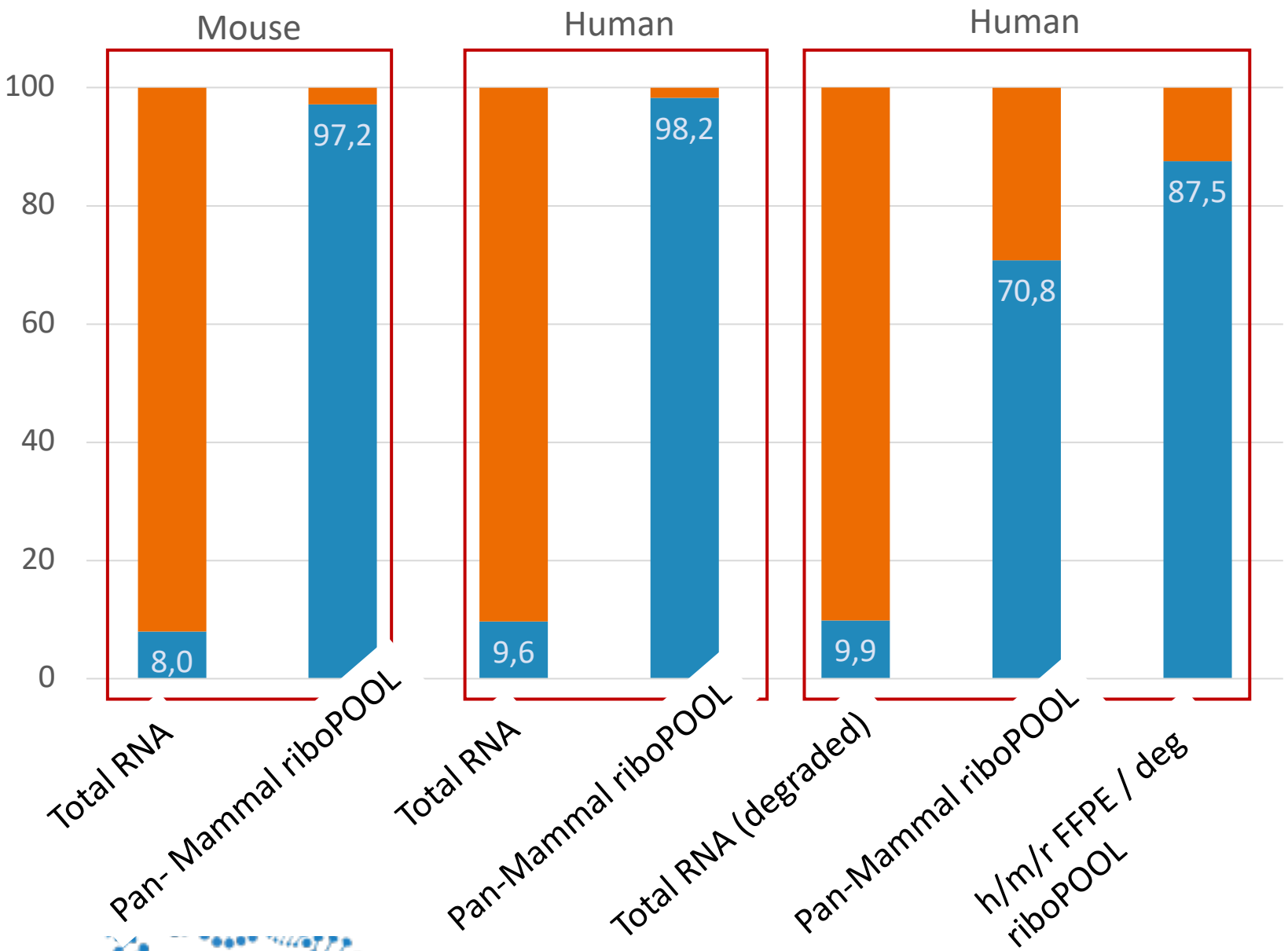
Experiment Conditions	
Input (ng)	1000
RIN	**~8**
RNA Isolation	QIAgen Rneasy
rRNA depletion	Pan-Plant riboPOOL Kit
RNA clean-up	RNA XPClean (Beckman Coulter)
Library prep	NEB Kit (modified)
Sequencing	Illumina HiSeq300

Pan-Mammal riboPOOL for Universal Mammalian rRNA Depletion



- Efficient rRNA depletion tool
- Broad coverage of mammalia
- For tissue & cell culture derived RNA
- Targets 28S, 18S, 5.8S & 5S rRNA
- Targets mitochondrial rRNA

Pan-Mammal riboPOOL efficient across Species



Experiment Conditions	
Input (ng)	1000 of human and mouse RNA
RIN	**~8 and deg RNA**
RNA Isolation	M&N
rRNA depletion	riboPOOL Kit
RNA clean-up	RNA XPClean (Beckman Coulter)
Library prep	NEB Kit (modified)
Sequencing	Illumina NovaSeq® 6000

Benefits of riboPOOLs

Complex pool & optimally designed oligos ensure high **specificity and efficiency**

- **Flexible and Fast design**
- **Allow detection of all RNAs**
- **Suitable for metatranscriptomics and mixed samples**
- **Broad RNA input range (10 ng – 3µg)**
- **Fast workflow & automatable**
- **Affordable**
- **HPLC purified**

Ryan. D et. al (2020) A high-resolution transcriptome map identifies small RNA regulation of metabolism in the gutmicrobe Bacteroides thetaiotaomicron. **nature communications** (20) 11

Kim, I et. al (2019) Efficient depletion of ribosomal RNA for RNA sequencing in planarians. **BMC Genomics** 20, 909

Galmozzi, C. V. et. al (2019) Selective ribosome profiling to study interactions of translating ribosomes in yeast. **Nat Protoc** 14, 2279–2317

riboPOOL Kit - Reagents up to Library Prep

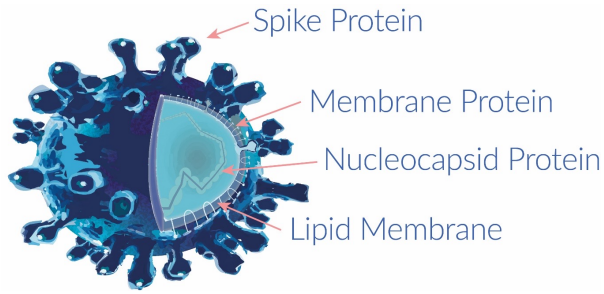
- Available in 6 (trial), 12, 24 and 96 reaction sizes
- Shipped freeze-dried at RT
- Complete with buffers, beads, ethanol clean-up reagents
- Probes and beads also available

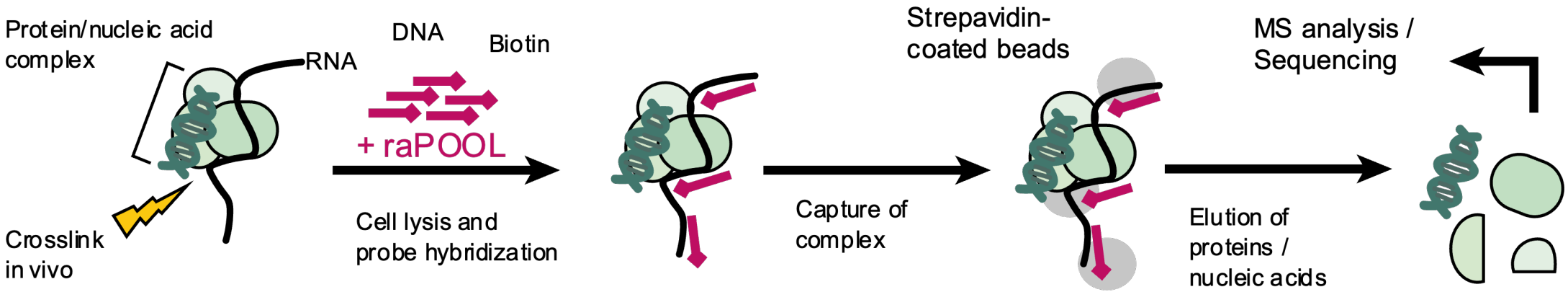
raPOOLs

For Robust & Targeted RNA Capture

raPOOLs – Targeted RNA Capture for Biochemical Analysis

- Study RNA properties
- Identify RNA for RNA modifications
- Identify & characterize RNA interacting partners

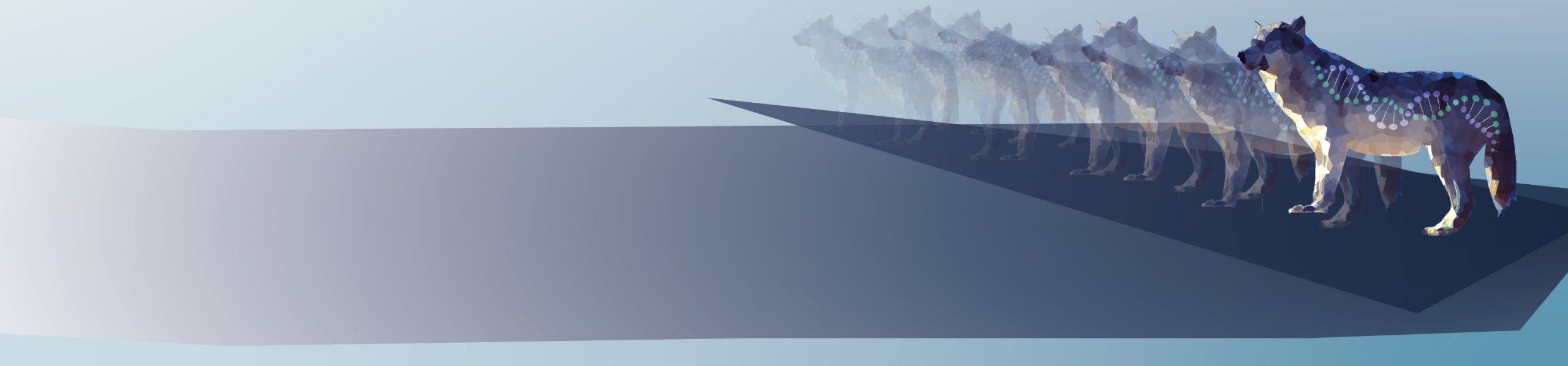




Nötzold, L. et. al The long non-coding RNA LINC00152 is essential for cell cycle progression through mitosis in HeLa cells. **Scientific Reports** 7, 2265 (2017)

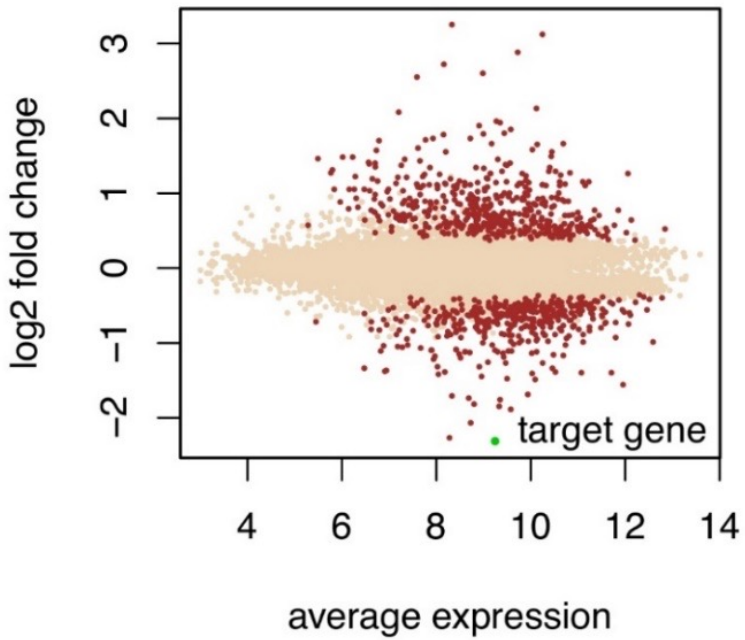
siPOOLs

For Reliable & Reproducible RNAi



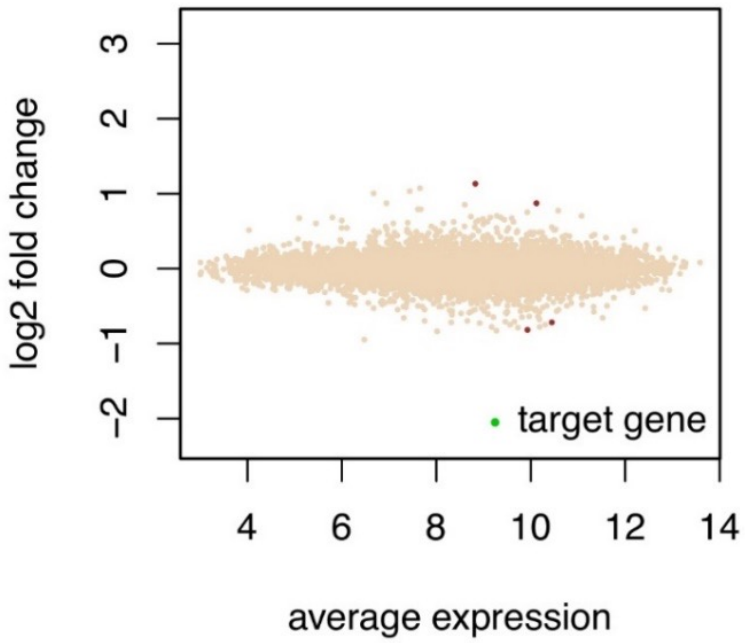
siPOOLs counter Off-target Effect in RNAi

Single siRNA



siRNA within siPOOL

siPOOL

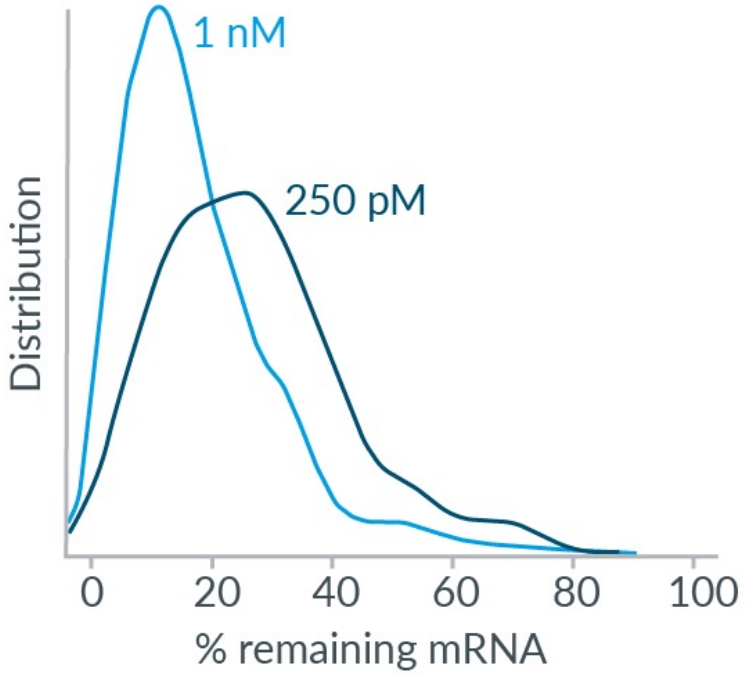


- *HeLa cells*
- *3 nM Scyl1 siRNA or siPOOL*
- *48 h*
- *whole transcriptome profiling by Affymetrix Microarray*

POOLing reduces off-target effects

Hannus et al., Nucleic Acids Res, 2014

siPOOL Efficient knockdown at low nano molar concentrations

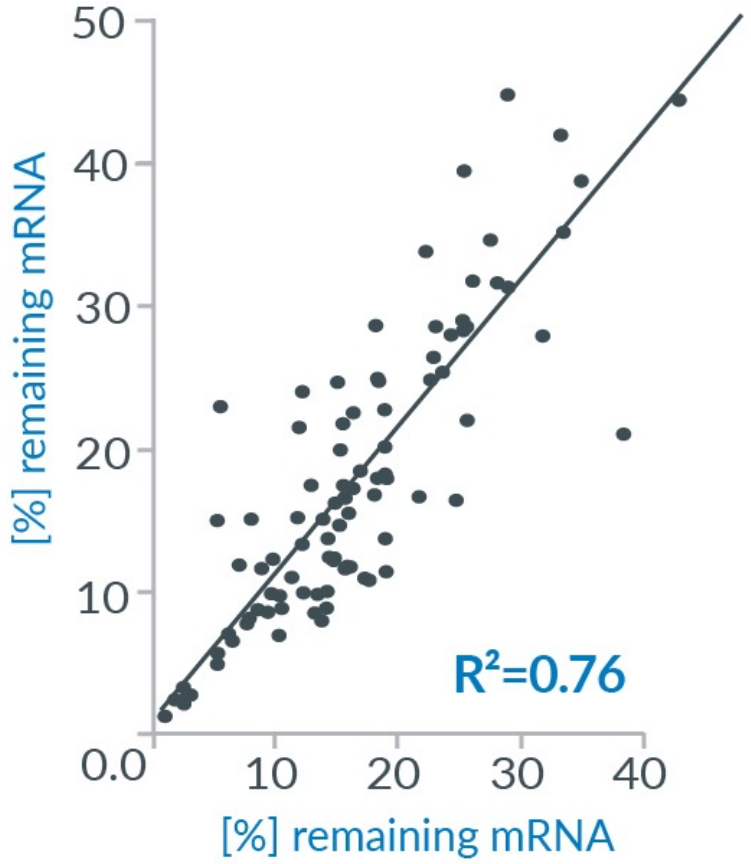


A549 SW48
HEK293 THP1
HUH7 HeLa
MCF7 HepG2

1 nM (708 siPOOLs)
250 pM (204 siPOOLs)

24 h

rt-qPCR



A549
HEK293
HUH7
MCF7
SW48

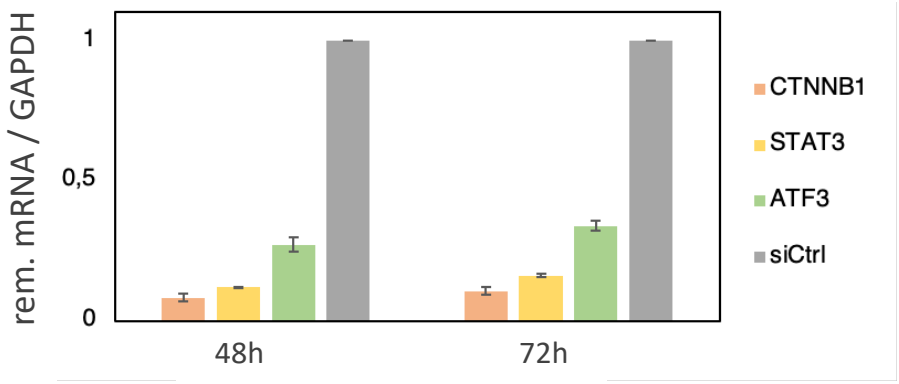
1 nM (96 genes)

24 h

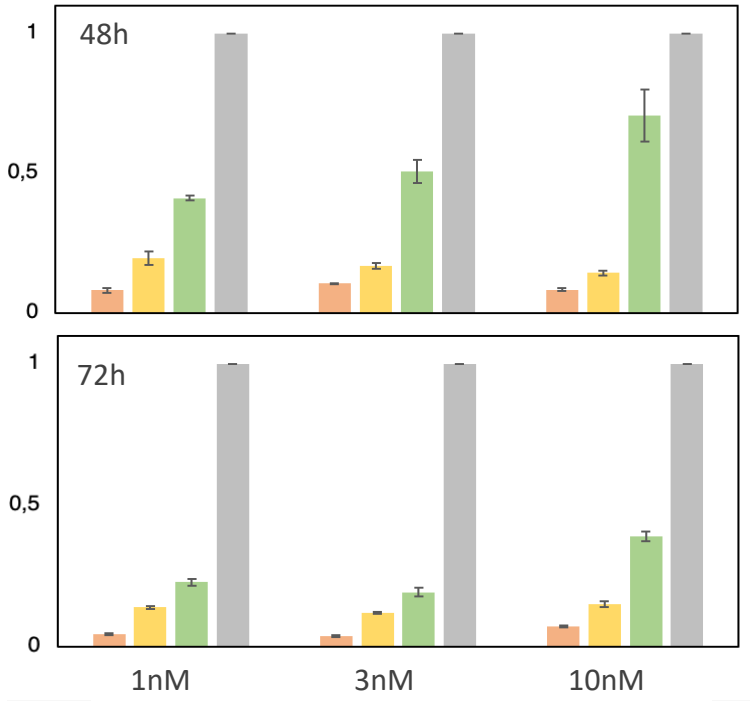
rt-qPCR

Case Study: triple target knock-down

single target knock down



triple target knock down



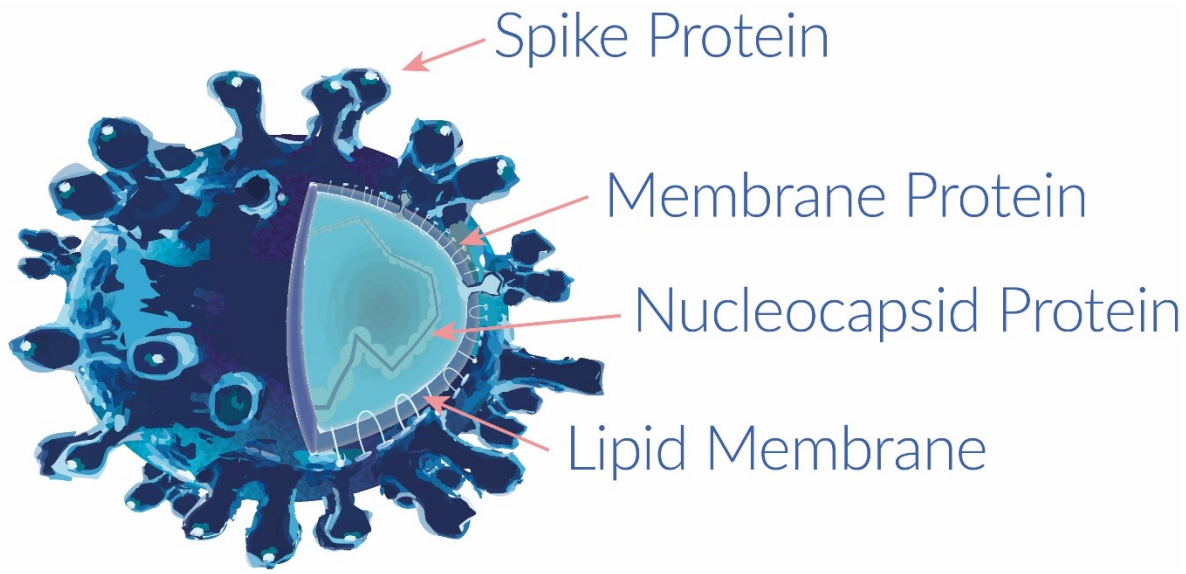
⇒ Triple target KD similar to single target KD

⇒ Even with triple target, 1nM saturating

data by **Michaela Bauer**, AG **Gunter Meister**, University of Regensburg, Germany

siPOOLs - SARS-CoV-2 Research

- RNAi Knockdown of human host factors
  - ACE2
  - TMPRSS2
- siPOOLs in combination



Thank You For Your Attention, Questions?

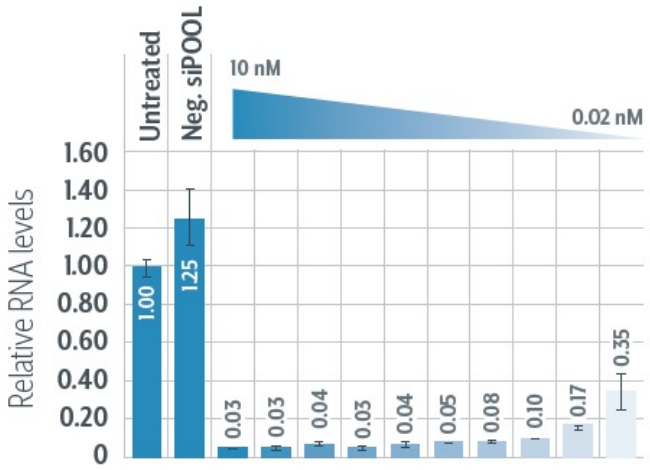
Contact us or your local distributor for questions/orders

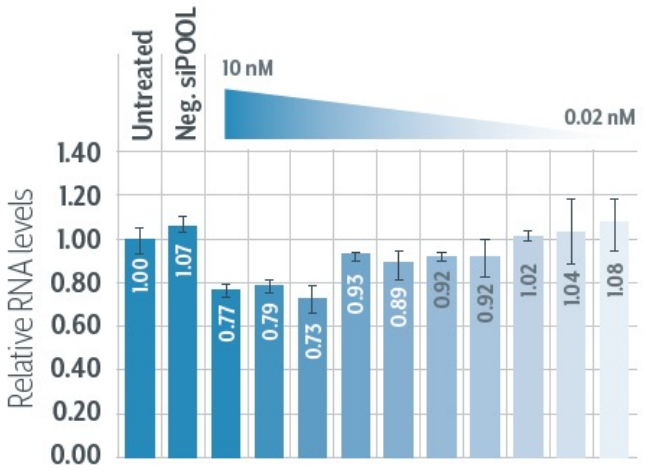
@siTOOLsBiotech Email: info@sitools.de

www.sitoolsbiotech.com

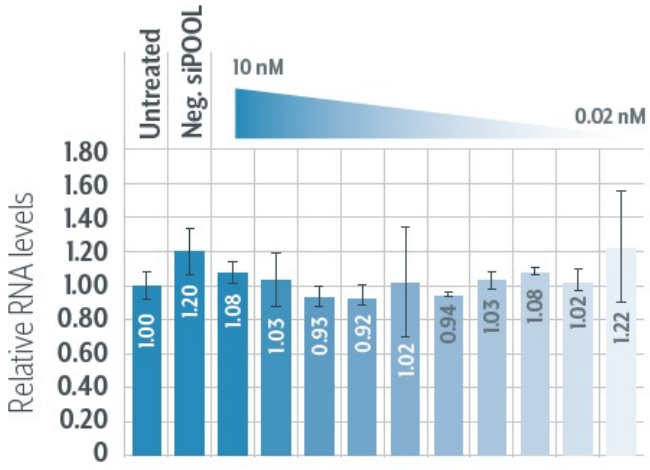
Increased Efficiency with siPOOL for Gene Silencing

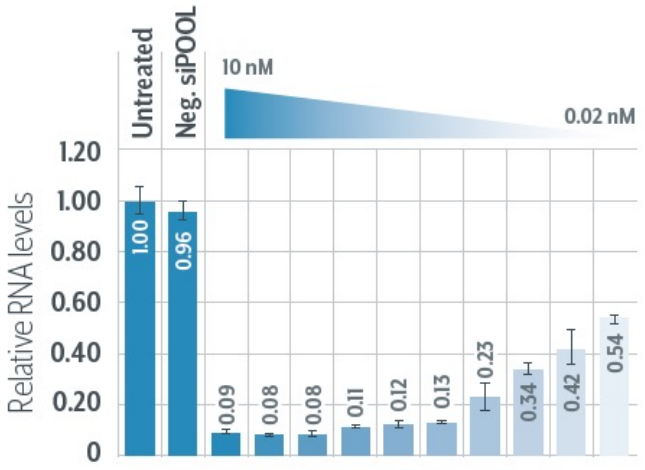
RT-PCR
SMARCA2





RT-PCR
SMARCA4

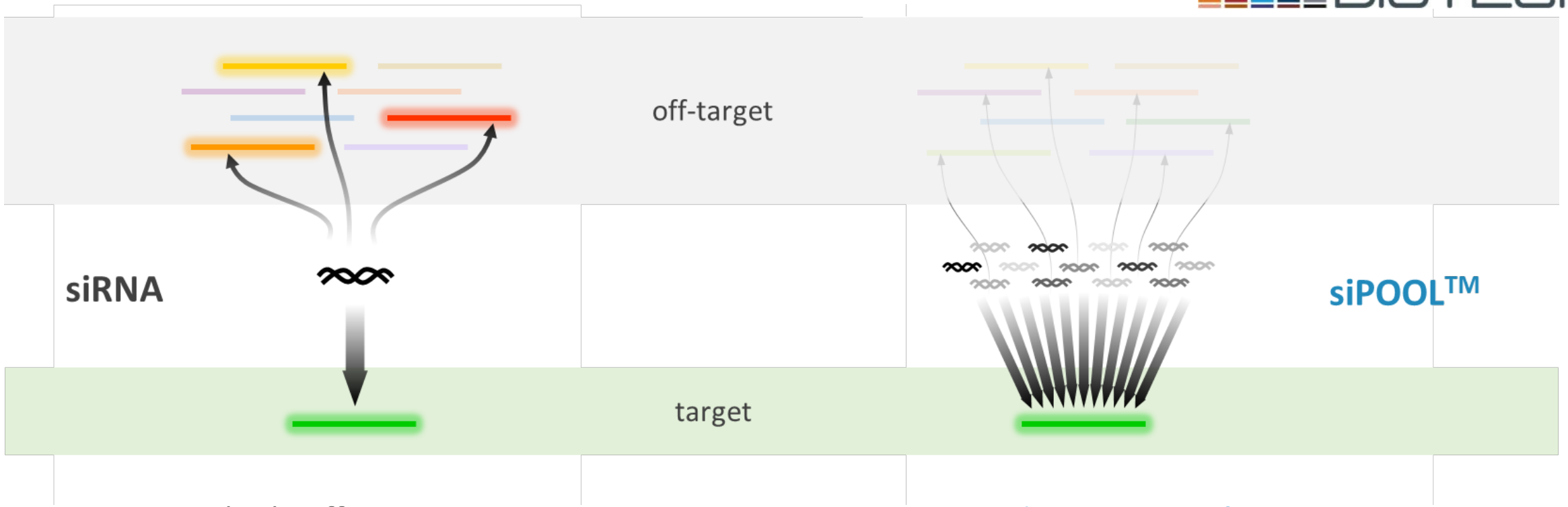




Dr. Mona Malz, PhD
Senior Scientist
Cancer Drug Discovery
German Cancer Research Center (DKFZ)
Heidelberg, Germany

dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

The Power of Complex POOLing



- Multiple off-targets
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- High target specificity
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