Comparative analysis of next-generation sequencing data generated from phage display trails with inorganic materials

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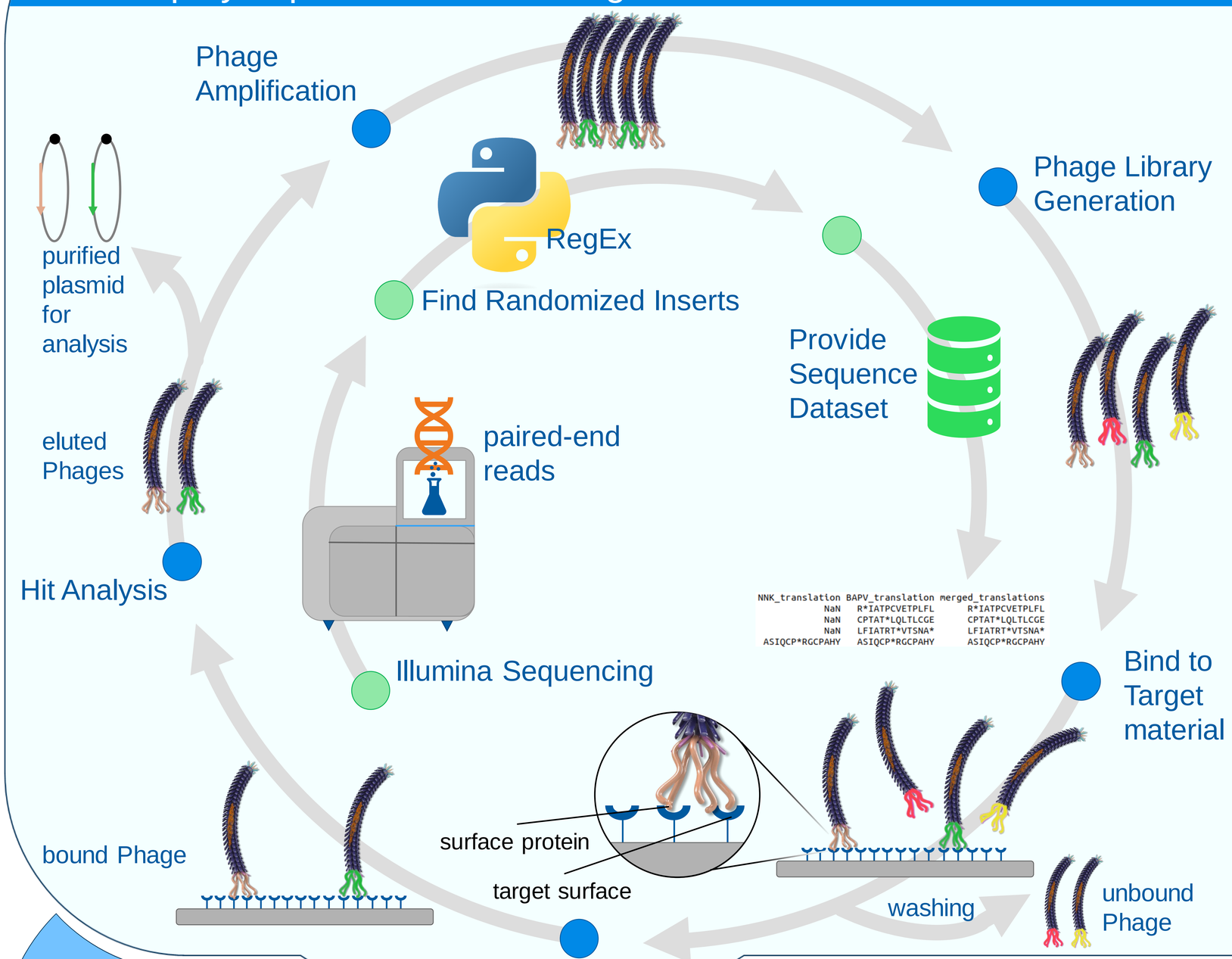
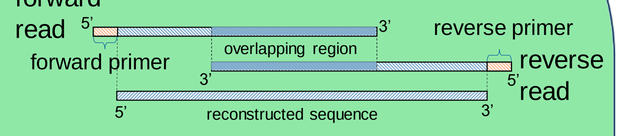
Objective: The aim of this work is to find meaningful and statistical proven ways to identify metal binding peptides in multiple trail phage display experiments with inorganic materials



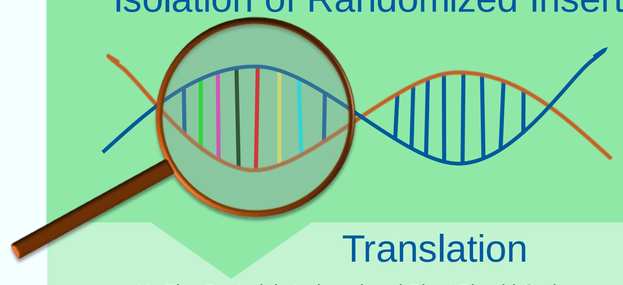
NNK_translation	BAPV_translation	merged_translations
NaN	R*IATPCVETPLFL	R*IATPCVETPLFL
NaN	CPTAT*LQLTLCGE	CPTAT*LQLTLCGE
NaN	LFIATRT*VTSNA*	LFIATRT*VTSNA*
ASIQCP*RGCPAHY	ASIQCP*RGCPAHY	ASIQCP*RGCPAHY

Prototype workflow

Joining Paired-End Reads



Isolation of Randomized Insert



Translation

11. The Bacterial, Archaeal and Plant Plastid Code

TTT F Phe	TCT S Ser	TAT Y Tyr	TGT C Cys
TTC F Phe	TCC S Ser	TAC Y Tyr	TGC C Cys
TTA L Leu	TCA S Ser	TAA * Ter	TGA * Ter
TTG L Leu i	TCG S Ser	TAG * Ter	TGG W Trp
CTT L Leu	CCT P Pro	CAT H His	CGT R Arg
CTC L Leu	CCC P Pro	CAC H His	CGC R Arg
CTA L Leu	CCA P Pro	CAA Q Gln	CGA R Arg
CTG L Leu i	CCG P Pro	CAG Q Gln	CGG R Arg
ATT I Ile i	ACT T Thr	AAT N Asn	AGT S Ser
ATC I Ile i	ACC T Thr	AAC N Asn	AGC S Ser
ATA I Ile i	ACA T Thr	AAA K Lys	AGA R Arg
ATG M Met i	ACG T Thr	AAG K Lys	AGG R Arg

Sequence data basis

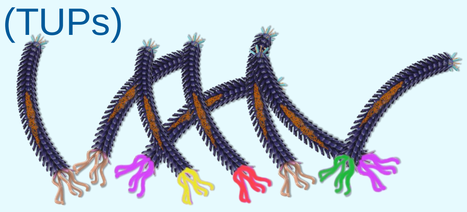
NNK_translation	BAPV_translation	merged_translations	NNK_type
EEHKCKKLMCLLTK	EEHKCKKLMCLLTK	EEHKCKKLMCLLTK	TRUE
	LFSLTRPLKT*VVH	LFSLTRPLKT*VVH	FALSE
	GL*QTD*CDTHSFF	GL*QTD*CDTHSFF	FALSE
DERRCGVQ*CWQMT	DERRCGVQ*CWQMT	DERRCGVQ*CWQMT	TRUE
	LLASTPQVITTVY*	LLASTPQVITTVY*	FALSE
	GL*QTD*CDTHSFF	GL*QTD*CDTHSFF	FALSE
RSR*CQHNYCRSTD	RSR*CQHNYCRSTD	RSR*CQHNYCRSTD	TRUE
*TVQCMRLMCSIAF	*TVQCMRLMCSIAF	*TVQCMRLMCSIAF	TRUE

vectorized data frames for faster data query

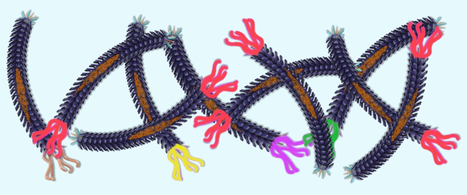
To Separate the Indistinguishable

- Difficult to distinguish between true binders and Target Unrelated Peptides (TUPs)

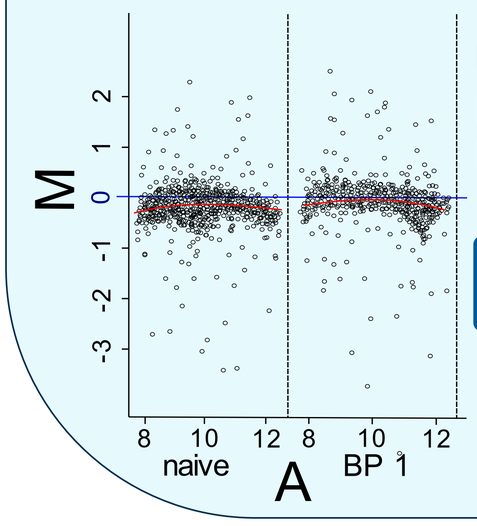
1. Round



2. Round



- Gradual increase of frequency in fast-propagating phage clones



Find fast propagating population

Statistical Methods to Discover Binding Peptides

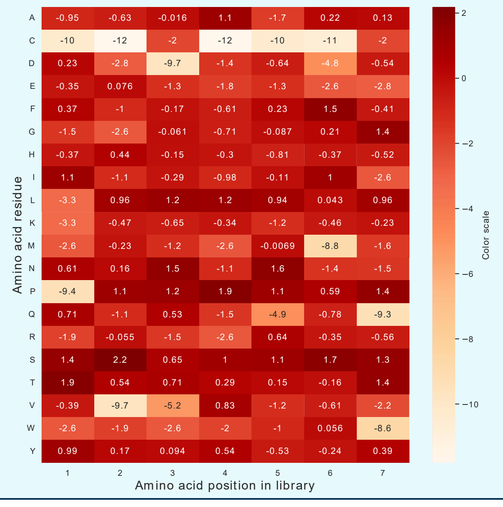
- Identifying TUPs with differential enrichment analysis[1]

Trimmed mean of M-values normalization and PCR

Testing of best fitting distributions and models

Checking false discovery rate and running statistical tests

- Identifying binding motifs with a derivative of the PepSimili[2] approach



Compare peptide motifs with protein domains using metal binding protein data base

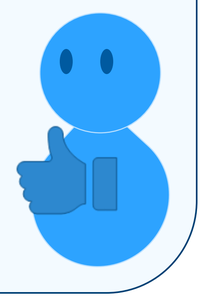
Future Outlook & Conclusion

Check optimal normalization methods

Consider data origin & experimental conditions !

Best tests for a multi-trail Phage display experiment !

Phage display trails are a complex evolution theory task with no current easy established solution to find best binders for inorganic materials



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[1] Robinson, M.D., Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 11, R25 (2010). https://doi.org/10.1186/gb-2010-11-3-r25
[2] Vekris A, Pilalis E, Chatziioannou A and Petry KG (2019) A Computational Pipeline for the Extraction of Actionable Biological Information From NGS-Phage Display Experiments. Front. Physiol. 10:1160. doi: 10.3389/fphys.2019.01160

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