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miR-CATCH identifies biologically active miRNA regulators of the pro-survival gene XIAP, in Chinese hamster ovary cells

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“miR-CATCH Identifies Biologically Active miRNA Regulators of the Pro-Survival Gene XIAP, in Chinese Hamster Ovary Cells”

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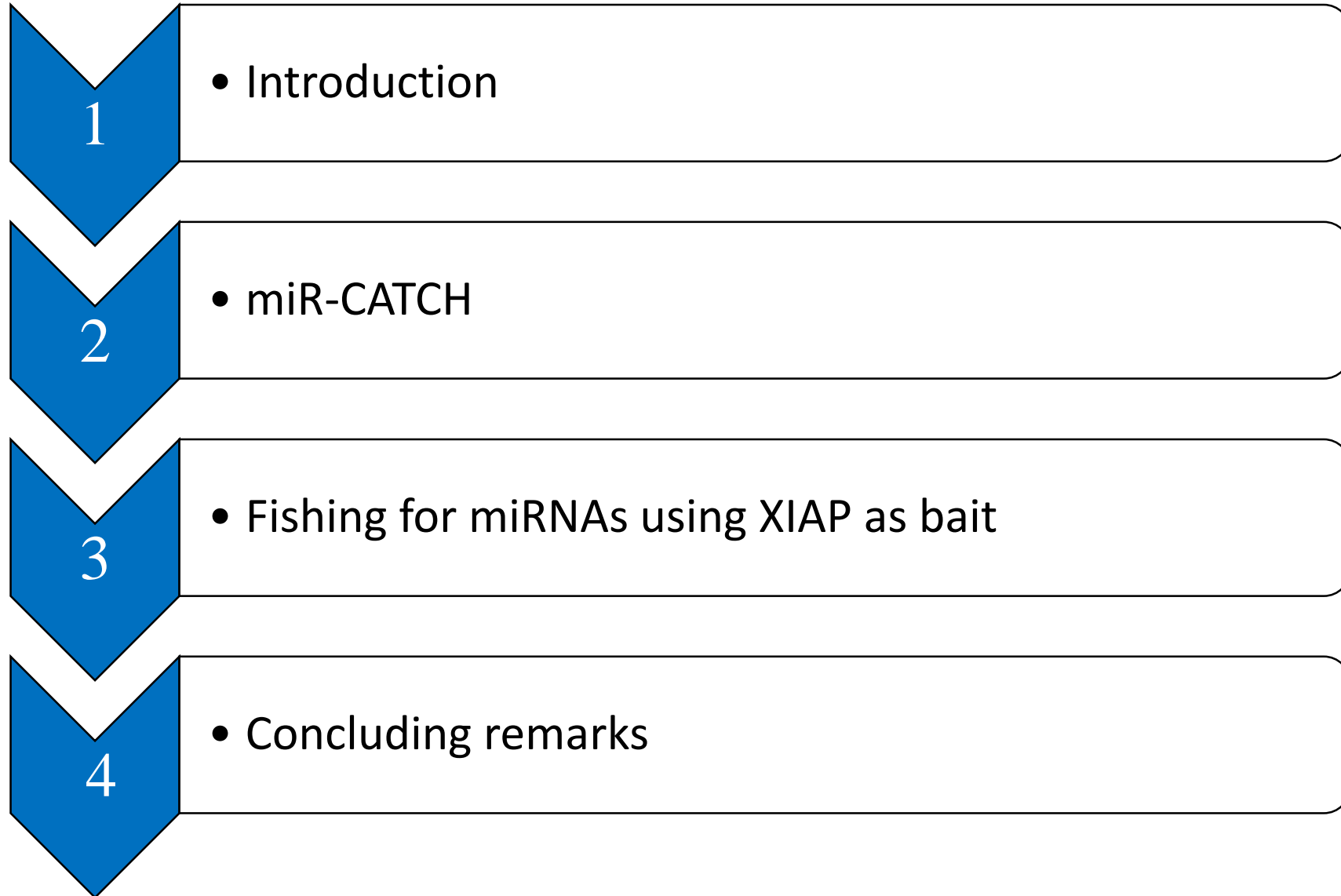
May 7th 2018

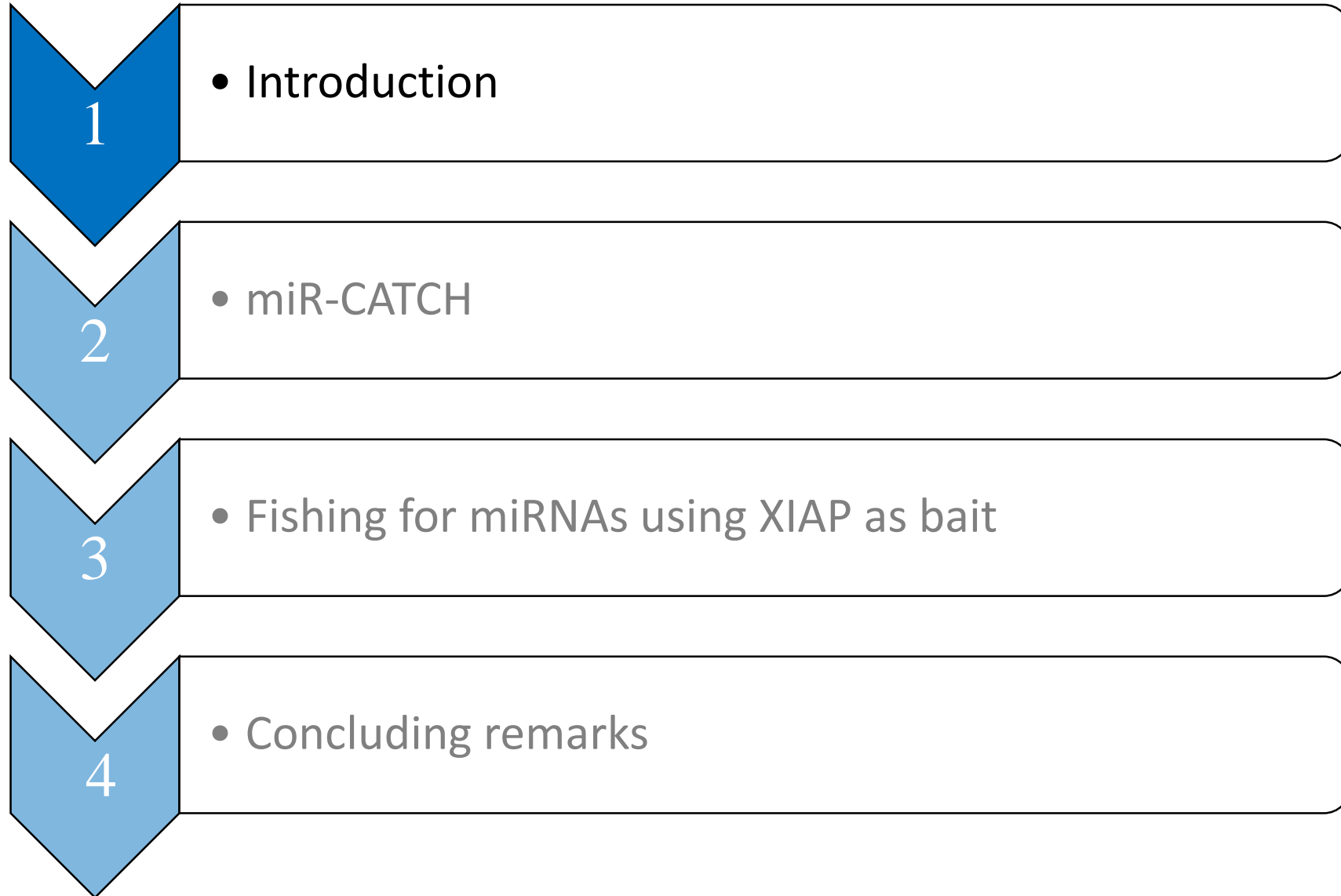


"BEFORE I CONTINUE, YOU'RE NOT ONE OF THOSE GUYS WHO BLAMES THE MESSENGER FOR BAD NEWS, ARE YOU?"

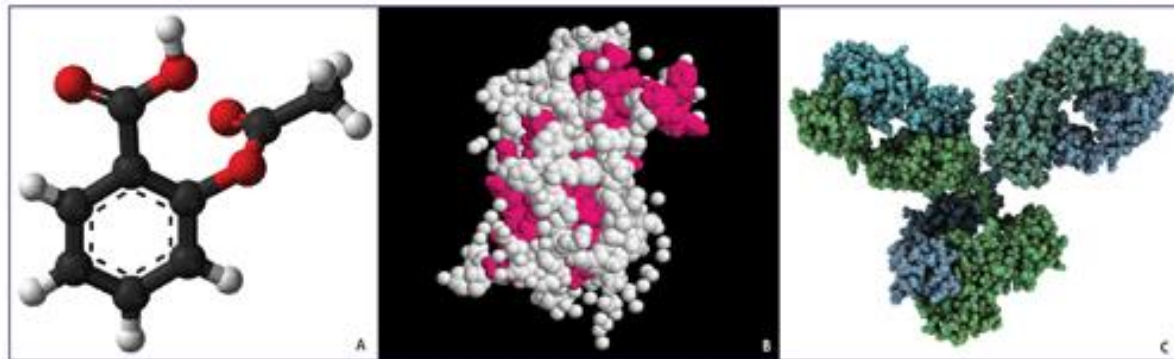


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- Dominant mammalian cell production host
- For 30 years we've been asking for MORE and MORE!!!
- Genetic engineering to push the boundaries



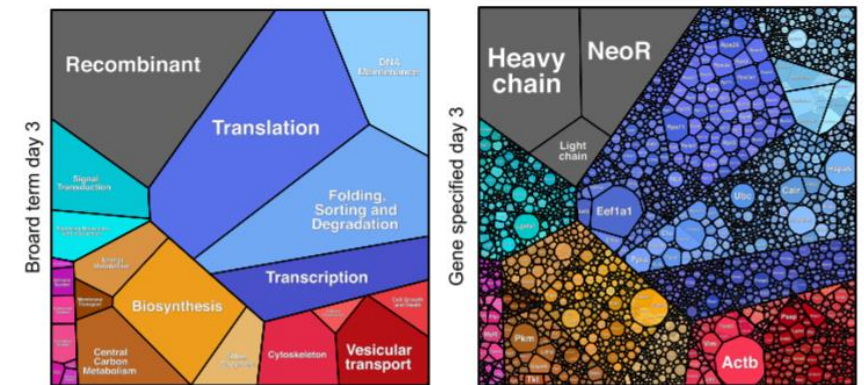
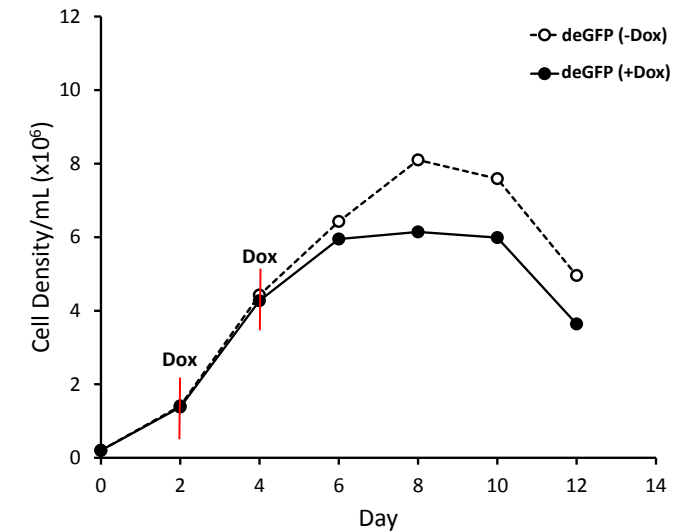
Aspirin
21 atoms
180 Da

Human Growth Hormone
~ 3,000 atoms
~ 22 kDa

IgG molecule
~ 20,000 atoms
~ 150 kDa

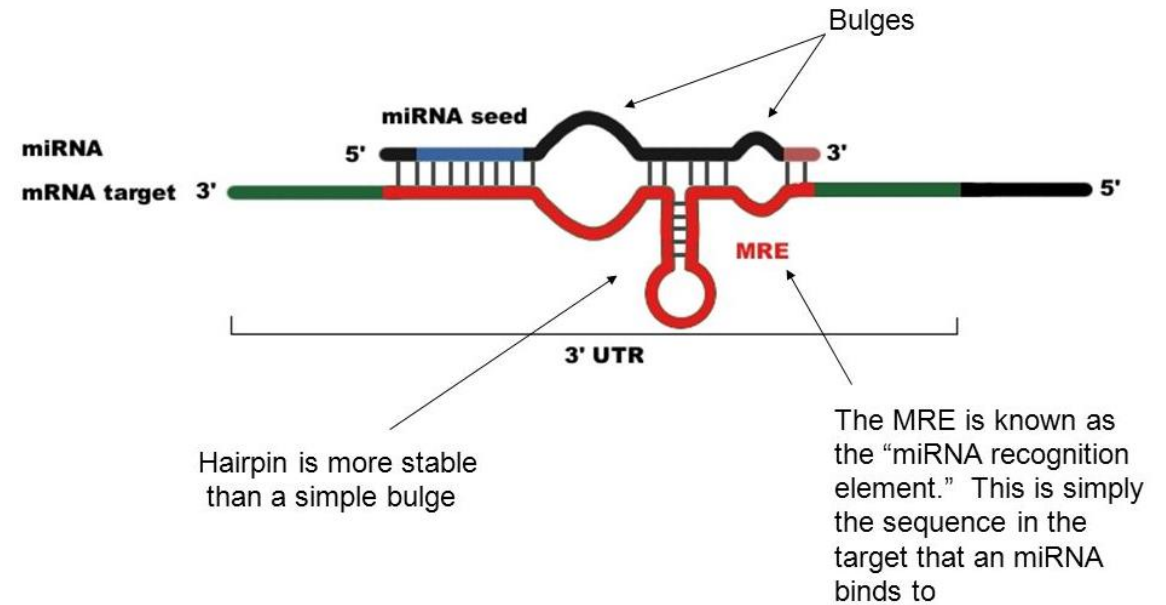


- Single/Multi-gene engineering in CHO
 - Over-expression
 - **Supraphysiological levels**
 - Compete for cellular resources
- Unwanted genes occupy translational space (e.g. NeoR)
 - Ribosomal Footprint Sequencing
- Over-expressed transgenes may achieve the anticipated Phenotype e.g. enhanced growth but can saturate the cells translational machinery
 - No titre benefits
 - Titre cost

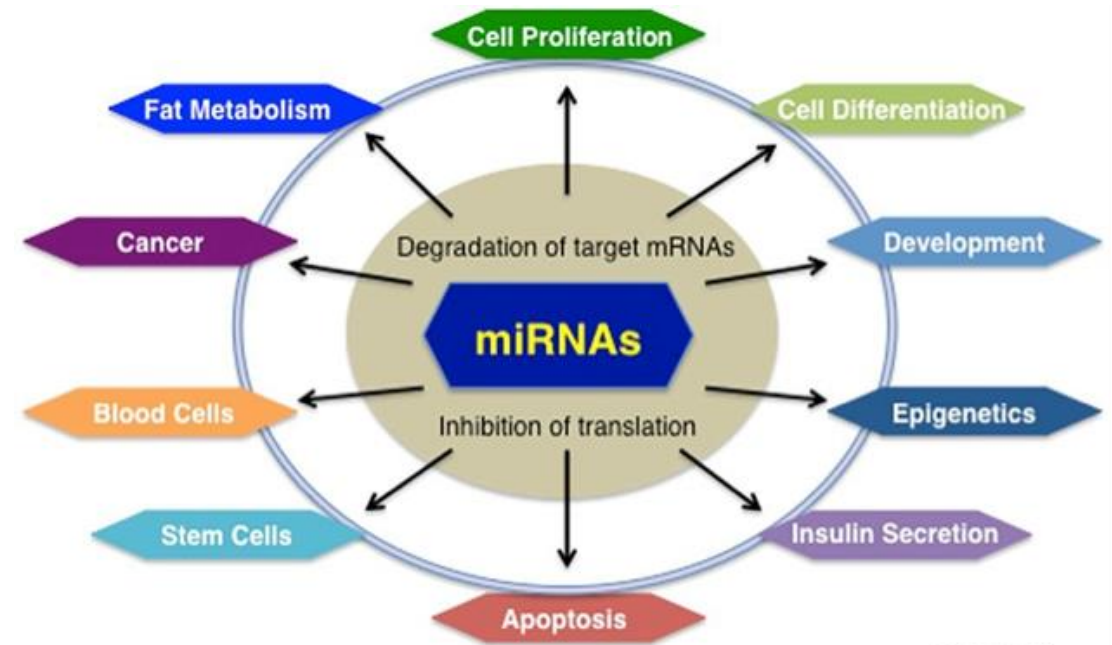


Kallehauge TB. et al. Scientific Reports. 2017, Jan 16; 7: 40388

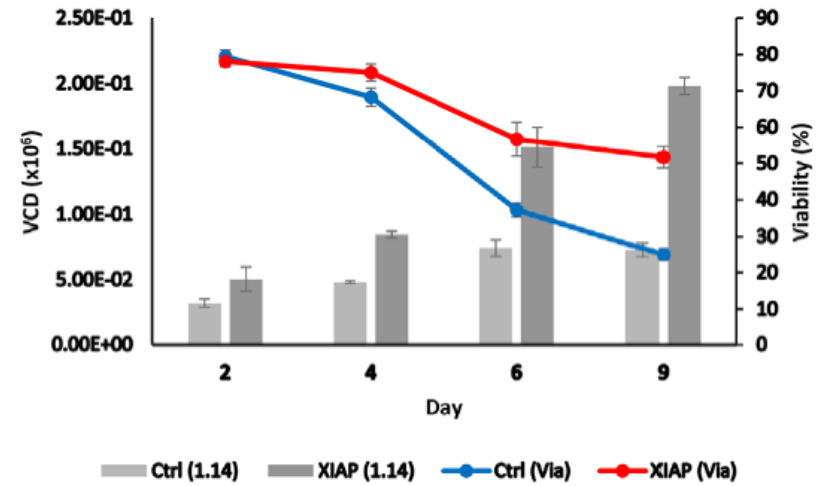
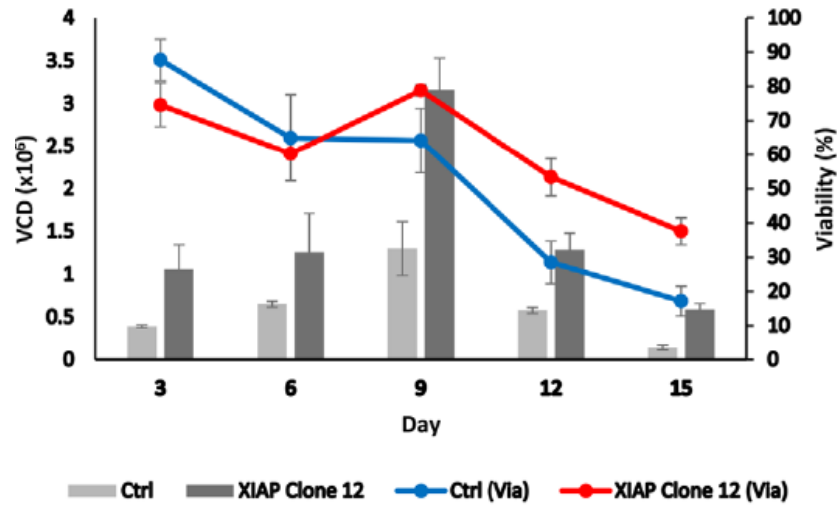
- Small non-coding RNAs (~ 22 nt)
 - Over-expression will not case a translational burden
- Post-translational regulation
 - 3'-UTR/CDS/5'-UTR
- Promiscuous little things
 - Seed binding
 - Imperfect complementarity
 - 10-100s of targets
- Potential to regulate multiple networks



- Numerous positive examples of miRNA engineering in CHO
 - miR-23, miR-7, miR-17, miR-143, miR-557, miR-2861 etc.
- Selected based on DE profiling
 - Growth, Viability etc.
 - Subsequent target identification, if at all
- Use more targeted approaches to regulate pre-validated genes
- X-linked inhibitor of apoptosis (XIAP)



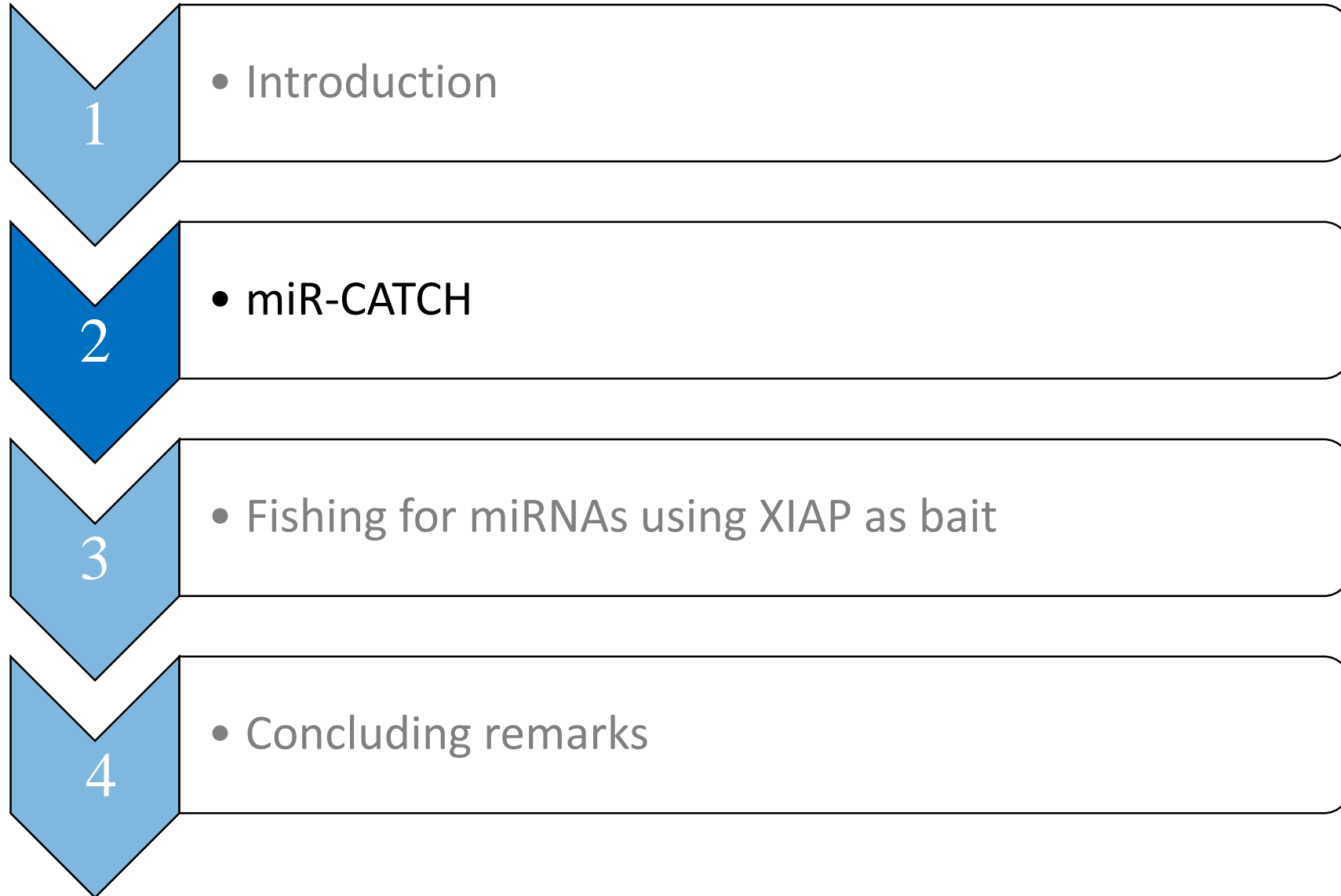
NaturemiRI

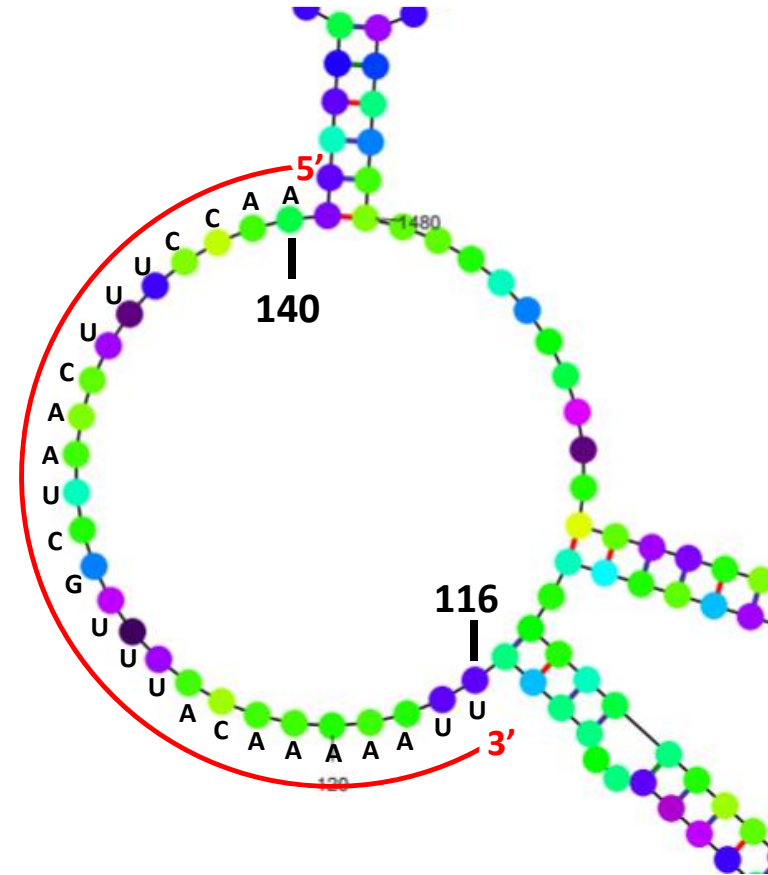
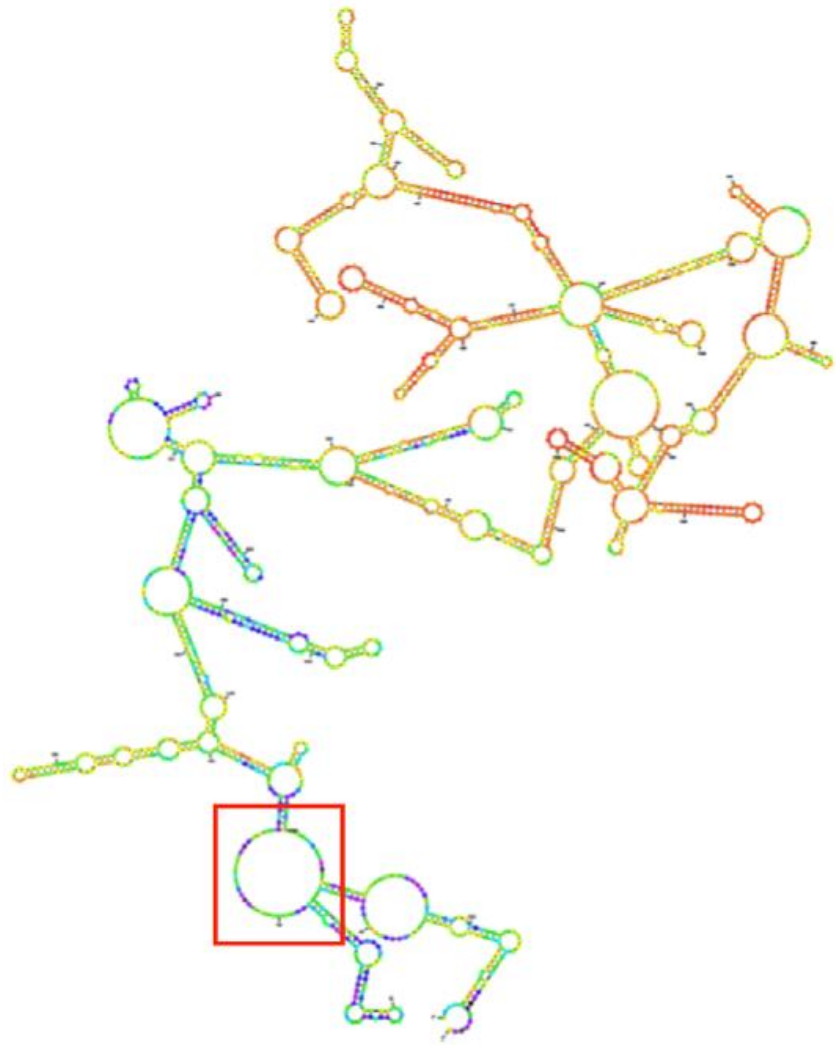


- Challenging selecting the right miRNA
- Non-canonical binding parameters
 - Target prediction unreliable
- Too many cooks spoil the broth
 - Unwanted target regulation
 - FUT8/miR-34a
- miR-CATCH

	Types	Base pairing	mRNA : miRNA	references
A Widespread canonical types	7-mer seed (2-8)	5' -AACUCACAACCAA CUCAGGGA -3' 3' AGUGUGAACUCCA GAGUCCU 5'	<i>lin-14</i> 3' UTR <i>lin-4 (C.elegans)</i>	Lee et al, 1993 Wightman et al, 1993
	6-mer seed (2-7)	5' -UAAGUUUCGUGUUGCAA GAACAAA -3' 3' AGUGC GCUCGGCUUG CUUGUUU 5'	<i>Mtpn</i> 3' UTR miR-375 (Mouse)	Poy et al, 2004
	offset 6-mer seed (3-8)	5' --UCA--AAA-- CUCAGGA --3' 3' AGUGUGAAACUCCA GAGUCCU 5'	<i>lin-14</i> 3' UTR <i>lin-4 (C.elegans)</i>	Lee et al, 1993 Wightman et al, 1993
B A few observed non-canonical types	near perfect site (3' compensatory)	5' -CCCAACAACAUGAA ACUGCCUA -3' 3' GGGUUGUUGUACUU UGAUGGAU 5'	<i>Hoxb8</i> 3' UTR miR-196 (Human)	Yekta et al, 2004
	3' compensatory site (bulge in seed)	5' -UUUUAUACAACCGUUCUAC A CUCA-3' 3' UUGAU AUGUUGG-AUG AUG GAGU-5'	<i>lin-41</i> 3' UTR <i>let-7 (C.elegans)</i>	Vella et al, 2004
	wobble in seed	5' -GUCUGAUUCAG-AA GGGCUCA -3' 3' UGUCCUAACUCCCC CCC GGGA 5'	<i>Nanog</i> CDS miR-296 (Mouse)	Tay et al, 2008
	seedless elements (mismatch in seed)	5' -GUGGGUGCU-CUGGG CUGAACCA -3' 3' GACA-AGGACGACUU GACUCGGU 5'	<i>E2f2</i> 3' UTR miR-24 (Human)	Lal et al, 2009
	centered site	5' -AGUUUU UCAGUCUGAUAA CUAU-3' 3' AGUUGU AGUCAGACUAUU CGAU 5'	<i>Gstm</i> 3' UTR miR-21 (Human)	Shin et al, 2010
C Widespread non-canonical types	nucleation bulge site	5' -CUCCUCA AUGUA GUG^GCCUUA -3' 3' CCGUAAGUGGCG CAC GGAU 5'	<i>Mink1</i> 3' UTR miR-124 (Human)	Chi et al, 2012
	seed-like motif	5' -UGGGGAUAGUGUUAU CGUAAU -3' 3' ACCCCUCAAU--GCGU GCUUUA 5'	<i>Gimap3</i> 3' UTR miR-155 (Mouse)	Loeb et al, 2012

Seok H et al. *Molecules and Cells*. 2016, 39(5): 375-381.



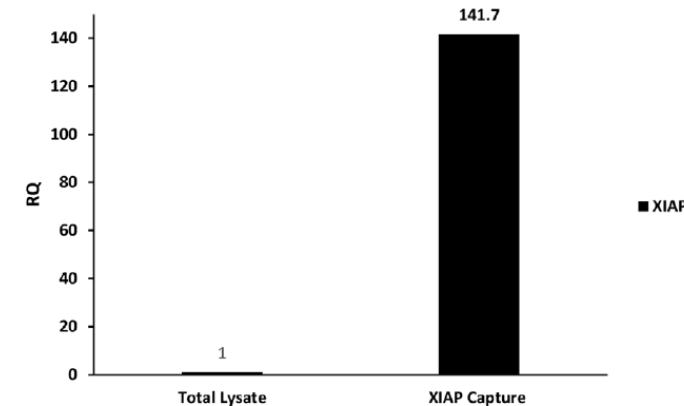
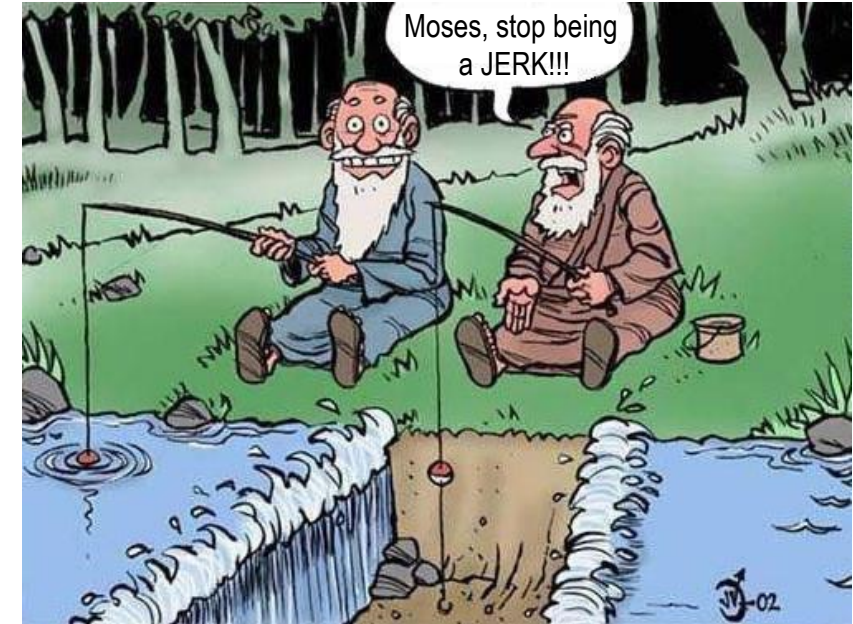
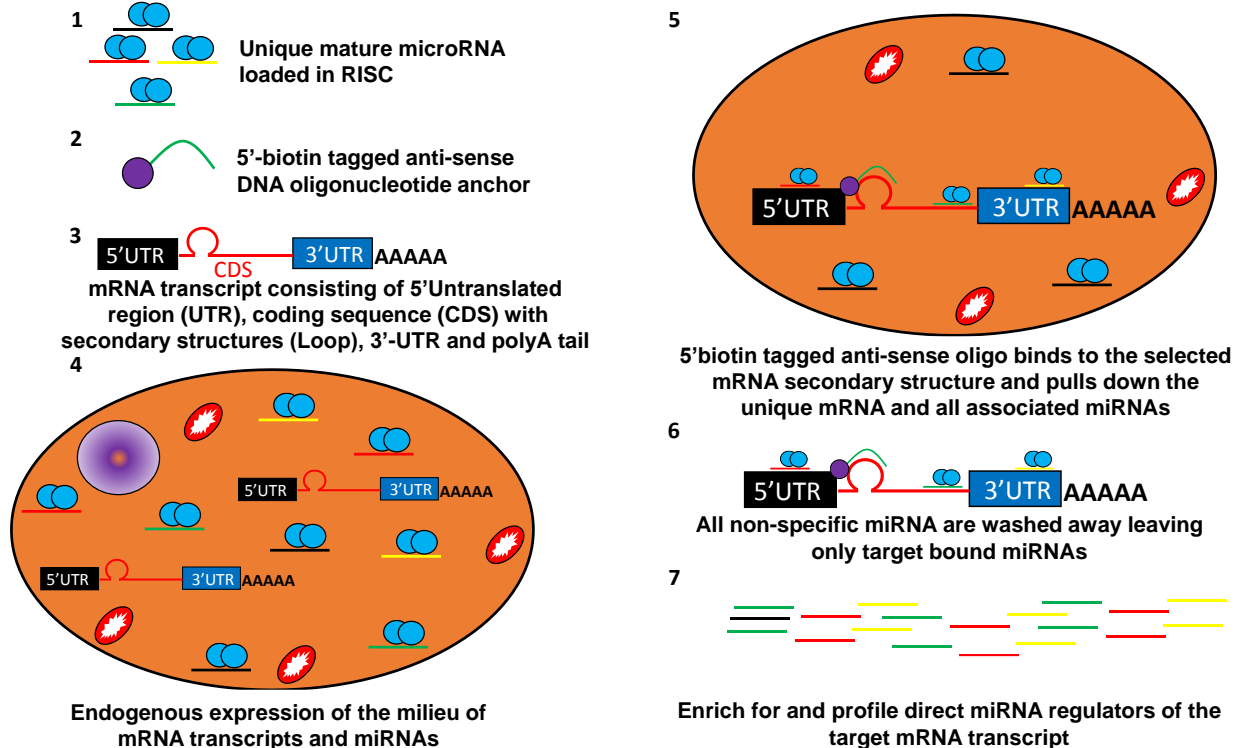


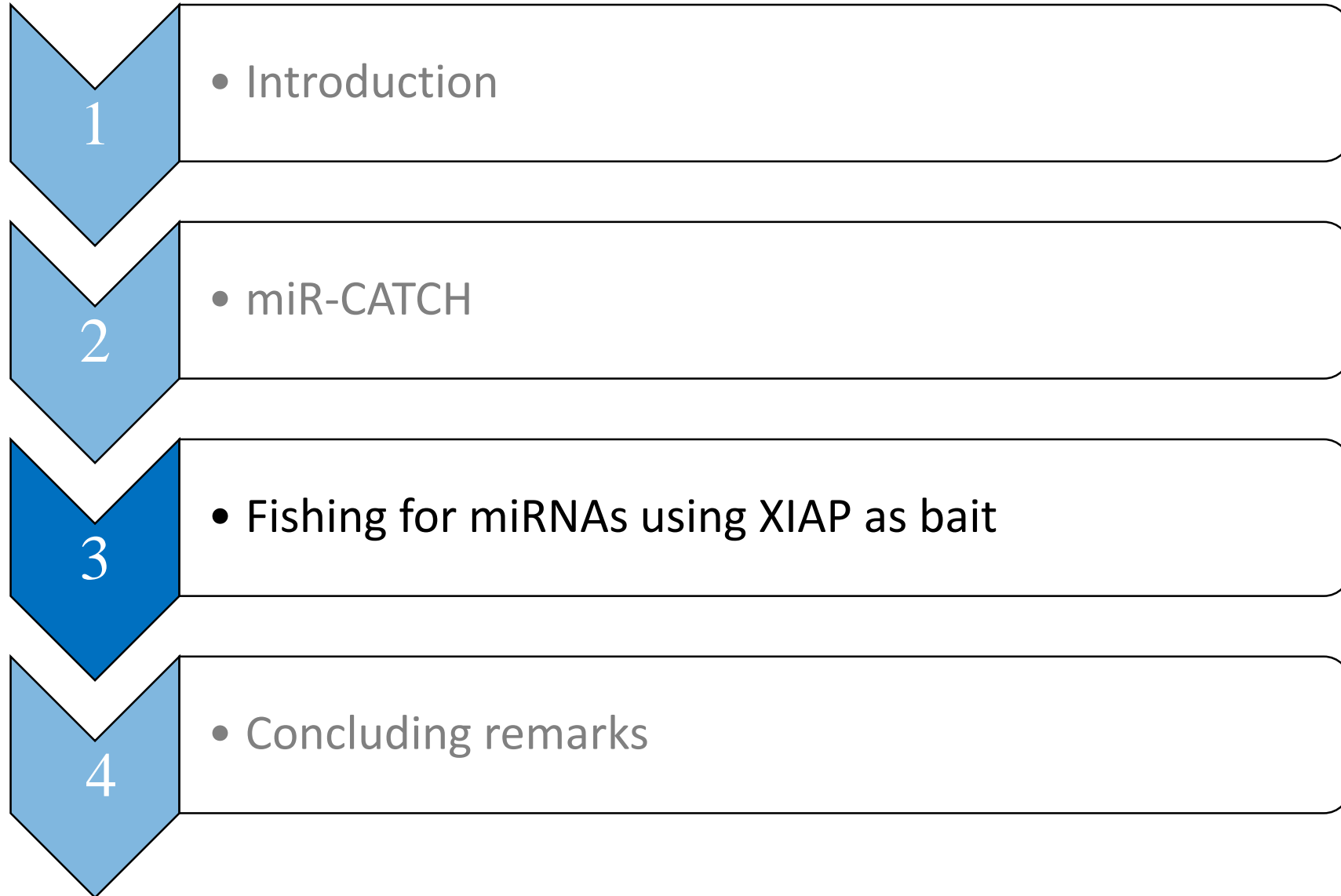
DNA Capture oligonucleotide
Biotin 5'-TTGGAAAGTTAGCAAATGTTTTTAA-3'

- Numerous pulldown methods:
 - Ago IP
 - Biotin-tagged miRNA mimics

miR-CATCH

Workflow



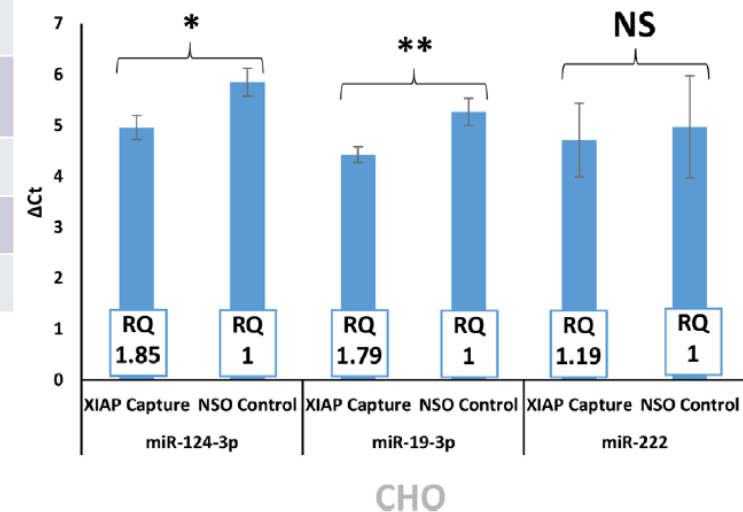


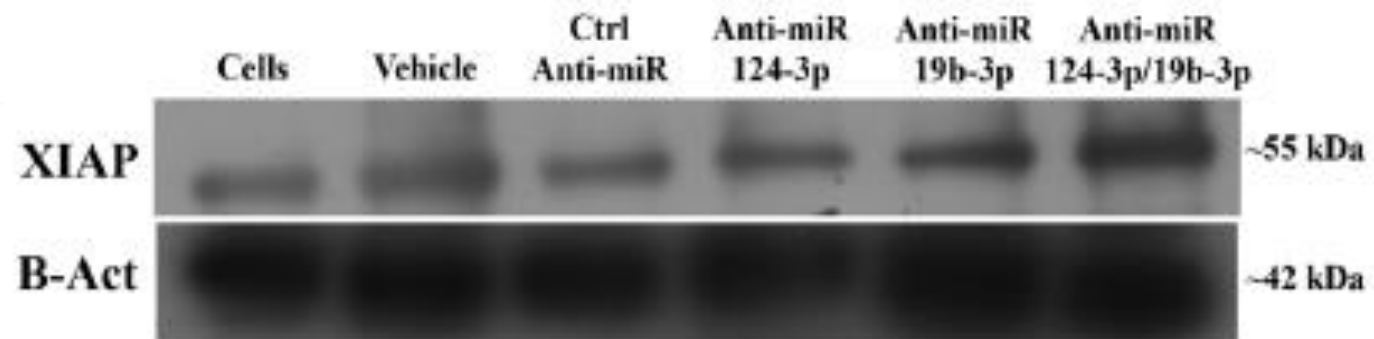
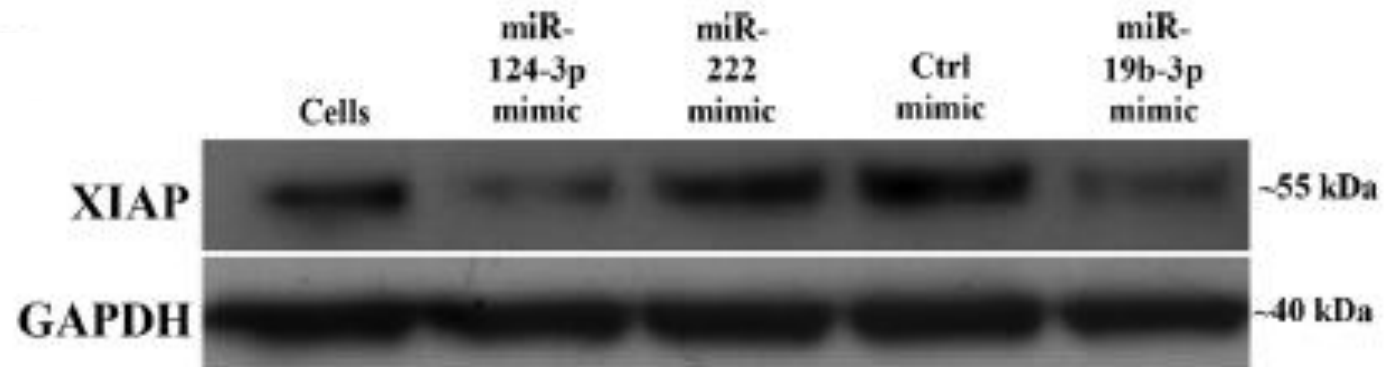
miRNA enriched in CHO XIAP-CATCH	<i>In-silico</i> predicted to be regulators of XIAP	miRNAs co-eluting with both CHO and Human XIAP
miR-17-5p	Y	N
miR-19b-3p	Y	N
miR-20a-5p	N	N
miR-30a-3p	Y	N
miR-30b-5p	Y	N
miR-30e-5p	Y	N
miR-124-3p	Y	Y
miR-125b-5p	Y	N
miR-125b-2-3p	Y	N
miR-526b-3p	Y	Y
miR-572	N	N
miR-610	Y	N
miR-760	Y	Y
miR-877-5p	Y	Y

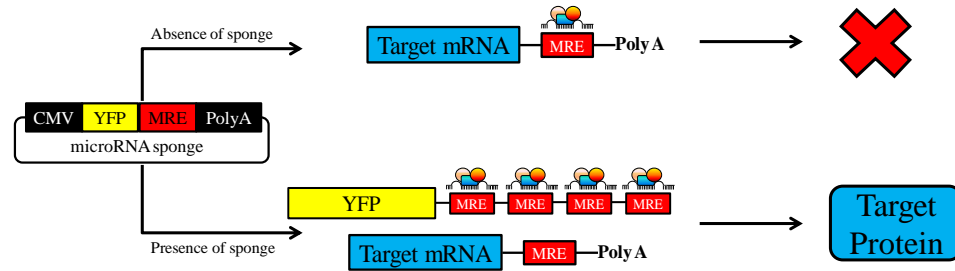
14 identified

26 identified

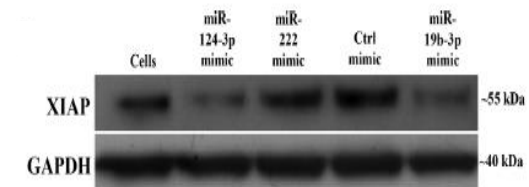
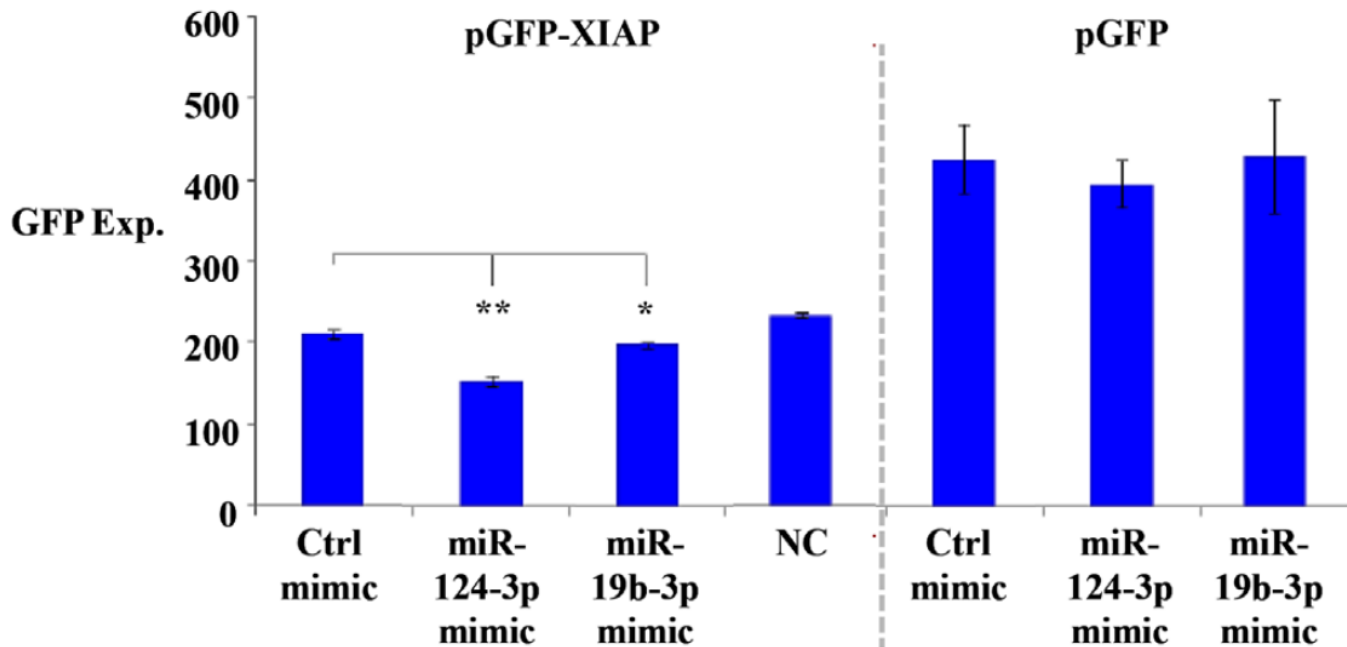
- 91% sequence similarity
 - Coding region
- 4 miRNAs overlapped
- 2 selected initial
 - miR-19b-3p
 - miR-124-3p







- Predominately 3'UTR
- GFP reporter “sponge”
- 3'UTR alone results in GFP repression
- miR-124-3p – binding sites within the 3'UTR
- miR-19b-3p – binding sites potentially within the coding sequence



- miR-124-3p/miR-19b-3p regulate XIAP translation
- 2 of 14 enriched miRNAs validated – 12 remaining
- Achieve XIAP overexpression through miR-124-3p/19b-3p knockout using CRISPR-Cas9
- Minimise translational burden
- Full catalogue of miRNA targets
 - Numerous cell lines
 - Numerous cell types
 - Culture stages
 - Increasing predictability

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