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BCOMING

D2.5 Report on standardised molecular assays

Project acronym: BCOMING

Project title: Biodiversity Conservation to Mitigate the risks of emerging infectious diseases

Call: HORIZON-CL6-2021-BIODIV-01

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TABLE OF CONTENTS

Disclaimer 3

Table of Contents 4

Executive Summary 5

Annex 1: coronavirus system evaluation 8

Annex 2: Coronasystems 12

Annex 3: Filoviruses 14

Annex 4 : HybridizationCapture_SOP overview 18

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EXECUTIVE SUMMARY

BCOMING is a multidisciplinary project that with partners and teams collecting samples from terrestrial and aquatic ecosystems, from humans and animals, in Cambodia, Cote d'Ivoire, Guinea and Guadeloupe. It is therefore very important to standardize sampling collection and subsequent laboratory analysis as much as possible. This deliverable concerns the evaluation and recommendation of PCR systems for analysis of coronaviruses and filoviruses from animal samples as well as of methods for the generation of full-length genomes of coronaviruses.

In-silico and bench evaluation of coronavirus PCR assays

A variety of commonly used PCR systems for detection of coronaviruses were assessed by HZI both in-silico, using the ecoPCR function from the package obitools (1), and by testing the performance of these assays in the lab using positive controls and bats' nucleic acids extracts.

These qualitative tests highlighted the suitability for coronavirus screening of multiple systems, but under the limited conditions tested the semi-nested system designed by Chu et al (2011), with cycling condition adapted as Lacroix et al (2020), seemed to outperform the others. It was therefore the system chosen by HZI, IRD and CERFIG for coronavirus screening in bat samples.

Given the large diversity of the coronavirus, other PCR assay might give better results for specific combinations of host species-virus. The systems used by IPC for coronavirus screening are the recommended two from the PREDICT consortium, which were also included in the in-silico and wet-lab tests performed by HZI, and gave good results as well.

Although we do not anticipate that this difference will translate in divergent estimates of coronavirus diversity, HZI will formally retest a sizable fraction of all their positive samples with the systems used by IPC.

A short summary of the assay comparison is reported in annex D2.5_annex1.

(1) Boyer, F., et al. (2016). "obitools: a unix-inspired software package for DNA metabarcoding." Mol Ecol Resour **16**(1): 176-182.
(2) Chu, D. K., et al. (2011). "Avian coronavirus in wild aquatic birds." J Virol **85**(23): 12815-12820.
(3) Lacroix, A., et al. (2020). "Wide Diversity of Coronaviruses in Frugivorous and Insectivorous Bat Species: A Pilot Study in Guinea, West Africa." Viruses **12**(8).

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PCR assays in use for coronavirus detection

A selection of assays are currently in use by different BCOMING partners. The assays are described in Lacroix, A., et al. (2020) for HZI, CERFIG and IRD, and by the PREDICT consortium in Anthony et al (2017). These assays are described in greater detail as SOP in the attached document D2.5_annex2.

(4) Anthony, S. J., et al. (2017). "Global patterns in coronavirus diversity." Virus Evol **3**(1): vex012.

PCR assays in use for filovirus detection

Different semi-nested assays are used by project partners to test for the presence of filoviruses.

HZI uses an unpublished semi-nested touch-down assay developed by Victor Corman (Institute of Virology, Charité, Berlin), targeting the RdRp, producing a fragment of 480 bp in the first reaction and a 428 bp fragment in the second reaction.

IRD and CERFIG use the semi-nested touch-down procotocol described by Goldstein et al (2018), where the L gene is targeted producing a 680 bp fragment in the first reaction and a 630 bp in the second. IPC uses a variation of this same assay, having the same primers but with different cycling conditions, as described by PREDICT consortium (modified from Zhai et al (2007)).

An overview of these protocols can be found in the attachement D2.5_Annex3_Filoviruses.

(5) Goldstein, T., et al. (2018). "The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses." Nat Microbiol **3**(10): 1084-1089.
(6) Zhai, J., et al. (2007). "Rapid molecular strategy for filovirus detection and characterization." J Clin Microbiol **45**(1): 224-226.

Coronavirus full genome sequencing via hybridization Capture

Analyses based on full coronavirus genomes can reveal important features of their evolution, notably by taking advantage of their complex recombination history, (Pekar et al, 2023). The generation and assembly of full genomes is therefore crucial if we are to unveil their origins. Enrichment of high-throughput sequencing libraries by hybridization capture using specific biotinylated baits is a flexible and cost-efficient approach to generate high-quality viral genomes. Given the challenges that this procedure presents, we developed different bait sets that allow for the enrichment of coronavirus sequences. Three bait sets were designed: a specific set targeting alphacoronavirus genomes, another one betacoronavirus genomes and a last one only targeting the spike gene of all coronaviruses (including alpha- and betacoronaviruses). The third bait set was designed to circumvent limited success in enriching this region with genomic bait sets, as revealed by initial

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attempts (a phenomenon also observed by Kuchinski et al, 2022). In addition, two commercial bait sets targeting a range of pathogens including human-infecting coronaviruses were also tested. The assessment performed at IRD on ca. 100 libraries assembled from positive bat samples showed that the recovery of near complete alpha- and betacoronavirus genomes is possible from >10% of the samples. The spike region drops out in nearly all cases, irrespective of the bait set used. The newly designed bait sets and one of the commercial bait sets performed similarly, the other commercial bait set exhibited lower efficiency. Based on these results, we recommend the use of one of the three aforementioned bait sets to assemble near complete genomes, complemented by PCR-based approaches to obtain the sequence of the spike region.
An overview of these protocols can be found in the attachment D2.5_Annex4_ HybridizationCapture_SOP. The detailed protocol is available as a .xls file upon request.

(7) Pekar, J., et al. (2023). “The recency and geographical origins of the bat viruses ancestral to SARS-CoV and SARS-CoV-2” bioRxiv 2023.07.12.548617; doi: https://doi.org/10.1101/2023.07.12.548617
(8) Kuchinski, K. S., et al. (2022). "Targeted genomic sequencing with probe capture for discovery and surveillance of coronaviruses in bats." Elife **11**. doi: 10.7554/eLife.79777

Transfer of technology and training

Hybridization-capture bait kit designed at HZI for enrichment of alphacoronavirus and betacoronavirus genome was shared with IRD. Subsequently the newly designed bait kit for enrichment of coronavirus spike protein was also made available to IRD. IRD staff received training on the performance of the hybridization capture protocol.

ANNEX 1: CORONAVIRUS SYSTEM EVALUATION

1) **In-silico primer evaluation**

The most commonly PCR system found in literature were tested in-silico against the entire viral nr refseq database using the ecoPCR function, from the obitools package. An overview of the primer use for these tests can be observed in table 1. A set of primers newly designed by Lagostina, here named “panCOV” was also included.

A different amount of base mismatches (e) was allowed, from 0 (perfect match) to 3 (relaxed match). The predicted amplification, simulated with ecoPCR, do not allow for a direct comparison between these systems by itself, since here we cannot correct for specific cycling conditions (conventional end point PCR vs semi/nested PCR, touch downPCR). Non-predicted are also possible interactions of the different PCR primers with the host genomes, which might led to unspecific amplification.

Nevertheless, a comparison between similar mismatching conditions, indicated how certain PCR systems were predicted to retrieve systematically higher amount of Coronavirinae taxa (Chu et al 2011, Lelli et al 2013 and panCOV). One of the three systems, Lelli, also was predicted to amplify a significant number of non-target viral sequence (20/63) when relaxed conditions were applied.

Comparison of primer sets via ecoPCR in−silico amplification

Genus: Alphacoronavirus, Betacoronavirus, Deltacoronavirus, Gammacoronavirus, Non Coronavirinae

e = number of allowed mismatches

nr of amplified taxa from viral nr refseq database

Primers used: Watanabe mod., † Chu, † Chu nest., * Gouilh, * Gouilh nest., Dominguez, † Lelli, panCOV, Poon, * Quan nest., Tong, Tong nest.

* touchdown PRC

† annealing temperature below 50°C

Figure 1. Histogram showing the number of coronavirinae taxa amplified in-silico with different PCR systems using the ecoPCR function from the obitools suite (Boyer et al 2016).

PRIMER NAME	SEQUENCE
panCov_A2_F	TGGGNTGGGAYTAYCCHAAGTG
panCov_A2_F_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGGGNTGGGAYTAYCCHAAGTG
panCov_B_R	GCNGTDGTDGCATCHCC
panCov_B_R_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCNGTDGTDGCATCHCC
Wat_mod_F	GGTTGGGAYTAYCCHAARTGTGA
Wat_mod_R_PCR_1&2	CCATCATCASWYRAATCATCATA
Wat_mod_F_NestA	GAYTAYCCHAARTGTGAYAGAGC
Wat_mod_F_NestA_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAYTAYCCHAARTGTGAYAGAGC
Wat_mod_F_NestB	GAYTAYCCHAARTGTGAUMGWGC
Wat_mod_F_NestB_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAYTAYCCHAARTGTGAUMGWGC
Wat_mod_R_PCR_1&2_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATCATCASWYRAATCATCATA
Chu_F	GGKTGGGAYTAYCCKAARTG

Chu_R	TGYTGTSWRCARAAYTCRTG
Chu_F_Nest	GGTTGGGACTATCCTAAGTGTGA
Chu_F_Nest_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTGGGACTATCCTAAGTGTGA
Chu_R_Nest	CCATCATCAGATAGAATCATCAT
Chu_R_Nest_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATCATCAGATAGAATCATCAT
Gou_F	GGTTGGGAYTAYCCWAARTGTGA
Gou_R_PCR_1&2	CCATCRTCMGAHARAATCATCATA
Gou_F_Nest	GCNAATWSTGTNTTTAACAT
Gou_F_Nest_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCNAATWSTGTNTTTAACAT
Gou_R_PCR_1&2_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATCRTCMGAHARAATCATCATA
Poon_F	GGTTGGGACTATCCTAAGTGTGA
Poon_F_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTTGGGACTATCCTAAGTGTGA
Poon_R	CCATCATCAGATAGAATCATCATA
Poon_R_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATCATCAGATAGAATCATCATA
Lelli_F	CDCAYGARTTYTGYTCNCARC
Lelli_F_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCDCAYGARTTYTGYTCNCARC
Lelli_R	RHGGRTANGCRTCWATDGC
Lelli_R_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGRHGGRTANGCRTCWATDGC
Quan_F	CGTTGGIACWAAYBTVCCWYTICARBTRGG
Quan_R	GGTCATKATAGCRTCAVMASWWGCNACNACATG
Quan_F_Nest	GGCWCCWCCHGGNGARCAATT
Quan_F_Nest_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCWCCWCCHGGNGARCAATT
Quan_R_Nest	GGWAWCCCCAYTGYTGWAYRTC
Quan_R_Nest_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGWAWCCCCAYTGYTGWAYRTC
Tong_F_PCR_1&2	ATGGGITGGGAYTATCCWAARTGTG
Tong_R	AATTATARCAIACAACISYRTCRTCA
Tong_F_PCR_1&2_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGGGITGGGAYTATCCWAARTGTG
Tong_R_Nest	CTAGTICCACCIGGYTTWANRTA
Tong_R_Nest_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTAGTICCACCIGGYTTWANRTA
Pauly_R_Nest	CCAACAYTTNGARTCWGCCAT
Pauly_R_Nest_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAACAYTTNGARTCWGCCAT
DSL_PC2S2_A	TTATGGGTTGGGATTATC
DSL_PC2S2_B	TGATGGGATGGGACTATC
DSL_PC2As1_C	TCATCACTCAGAATCATCA
DSL_PC2As1_D	TCATCAGAAAGAATCATCA
DSL_PC2As1_E	TCGTCGGACAAGATCATCA
DSL_PCS_NestF1	CTTATGGGTTGGGATT ATCCTAAGTGTGA
DSL_PCS_NestF1_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTATGGGTTGGGATT ATCCTAAGTGTGA
DSL_PCS_NestF2	CTTATGGGTTGGGATTATCCCAAATGTGA
DSL_PCS_NestF2_miseq	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTATGGGTTGGGATTATCCCAAATGTGA
DSL_PCNAs_Nest_R	CACACAACACCTTCATCAGATAGAATCATCA
DSL_PCNAs_Nest_R_miseq	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACACAACACCTTCATCAGATAGAATCATCA

Table 1. List of coronavirus PCR primers tested.

2) Wet lab evaluation

PCR systems tested in-silico were also tested in real life conditions, used as screening systems on OC43 positive controls serial dilutions and cDNA obtained from bat intestine. The results were in general corroborating the prediction made using ecoPCR. From these tests Chu et al 2011 resulted to have superior sensitivity compared to other primer sets used, being able to repeatedly amplify coronavirus OC43 positive control at lower dilutions compared to other systems (see bottom right of figure 2).

These results might be due to a better specificity of Chu et al 2011 towards the OC43 sequence, but overall this primer set seems to be a suitable candidate for screening diverse and unknown coronaviruses. Furthermore, these primers are already the system of choice of IRD (with minor

modification in the cycling condition described by Lacroix et al 2020) and HZI, which has used them in the BIODIVERSA funded project Biodiv-Afreid. This modified protocol was therefore the selected one for coronavirus screening in animal samples for BCOMING.

Figure 2. Example of results obtained in the comparison of different PCR systems.

Boyer, F., et al. (2016). "obitools: a unix-inspired software package for DNA metabarcoding." Mol Ecol Resour **16**(1): 176-182.

Chu, D. K., et al. (2011). "Avian coronavirus in wild aquatic birds." J Virol **85**(23): 12815-12820.

Lelli, D., et al. (2013). "Detection of coronaviruses in bats of various species in Italy." Viruses **5**(11): 2679-2689.

Lacroix, A., et al. (2020). "Wide Diversity of Coronaviruses in Frugivorous and Insectivorous Bat Species: A Pilot Study in Guinea, West Africa." Viruses **12**(8).

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ANNEX 2: CORONASYSTEMS

PCR Assays in use for pan-Coronaviruses by HZI, IRD and CERFIG

Corona_Chu2011_TD

Chu 1 of 2

date: 25/07/2023

performed by: **Operator** Targe t: RdRp

Primer_F	**Chu_F**	GGKTGGGAYTAYCCKAARTG
Primer_R	**Chu_R**	TGYTGTSWRCARAAYTCRTG

PCR	1x	**Ansat z:**	1	+7,5 %
H2O	10.35	µL	10.4 µL	11.1 µL
10x Rxn Buffer	2.50	µL	2.5 µL	2.7 µL
MgCl2	1.00	µL	1.0 µL	1.1 µL
dUTPs	2.00	µL	2.0 µL	2.2 µL
Forward Primer	2	µL	2.00 µL	2.15 µL
Reverse Primer	2	µL	2.00 µL	2.15 µL
PlatiniumTaq	0.15	µL	0.15 µL	0.16 µL
Mix Vol	20.00	µL	20.0 µL	21.5 µL
cDNA	5.00	µL		
Total Vol	25.00	µL		

Temp.	**t**	**X**
95	5 min	
95	15 s	40
48	30 s	
72	50 s	
72°C	1 min	sec

expected length: ~602 bp

Primer_F	Chu_F_Nest	GGTTGGGACTATCCTAAGTGTGA
Primer_R	Chu_R_Nest	CCATCATCAGATAGAATCATCAT

PCR	1x	Ansatz:	1	+7,5 %
H2O	15.35	µL	15.4 µL	16.5 µL
10x Rxn Buffer	2.50	µL	2.5 µL	2.7 µL
MgCl2	1.00	µL	1.0 µL	1.1 µL
dNTPs	2.00	µL	2.0 µL	2.2 µL
Forward Primer	1	µL	1.00 µL	1.08 µL
Reverse Primer	1	µL	1.00 µL	1.08 µL
PlatiniumTaq	0.15	µL	0.15 µL	0.16 µL
Mix Vol	23.00	µL	23.0 µL	24.7 µL
cDNA	2.00	µL		
Total Vol	25.00	µL		

Temp.	t	X
95	5 min	
95	15 s	10 TD (-0.5°C each cycle)
53	30 s	
72	30 s	
95	15 s	35X
53	30 s	
72	30 s	
72°C	1 min	sec

expected lenght: ~440 bp

Lacroix, A., et al. (2020). "Wide Diversity of Coronaviruses in Frugivorous and Insectivorous Bat Species: A Pilot Study in Guinea, West Africa." Viruses 12(8).

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ANNEX 3: FILOVIRUSES

PCR Assays in use for pan-Filoviruses by HZI

Pan_Filo screening nested PCR

Date: ######## Corman et al, unpublished

performed by: operator Target region: RdRp fragment: 480nt (1st round) and 428nt (2nd round)

PanFiloVMC_F2	GCNTTYCCNAGYAAYATGATGGT
PanFiloVMC_R1	TGTNATRCAYTGRTTRTCNCC
PanFiloVMC_F3	TATTGCAYCARGCNTCNTGGCA

PCR 1 of 2	1x	unit	1	+5%
H2O	10.90	µL	11 µl	11 µl
10 x Rxn Puffer	2.50	µL	2.5 µl	3 µl
MgCl2	0.40	µL	0.4 µl	0 µl
BSA (1 mg/ml)	1.00	µL	1.0 µl	1 µl
dUTP's	1.00	µL	1.0 µl	1 µl
PanFiloVMC_F2	2.00	µL	2.0 µl	2 µl
PanFiloVMC_R1	2.00	µL	2.0 µl	2 µl
PlatinumTaq	0.20	µl	0.2 µl	0 µl
Mix Vol.	**20.00**	**µL**	**20 µl**	**21 µl**
DNA	**5.00**	µL		
Total Vol.	**25.00**	µL		

Temp.	t	X
95 °C	3 min	
94 °C	15 s	*10x TD (-1°C ea. Cyc.)
60 °C *	20 s	
72 °C	45 s	
95 °C	15 s	40X
50 °C	20 s	
72 °C	45 s	
72 °C	2 min	
8 °C	~	

expected length: 480 bp

PCR 2 of 2	1x	unit	1	+5%
H2O	18.15	µL	18 µl	19 µl
10 x Rxn Puffer	2.50	µL	2.5 µl	3 µl
MgCl2	1.25	µL	1.3 µl	1 µl
dUTP's	0.50	µL	0.5 µl	1 µl
PanFiloVMC_F3	0.75	µL	0.8 µl	1 µl
PanFiloVMC_R1	0.75	µL	0.8 µl	1 µl

Temp.	t	X
95 °C	3 min	
94 °C	15 s	*10 X TD (-1°C ea. Cyc.)
60 °C *	20 s	
72 °C	45 s	
95 °C	15 s	40X
50 °C	20 s	

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PlatinumTaq	0.10	µl	0.1 µl	0 µl
Mix Vol.	24.00	µL	24 µl	25 µl
1st Rd Product	**1.00**	µL		
Total Vol.	**25.00**	µL		

72 °C	45 s	
72°C	2 min	
8 °C	~	

expected length:	428 bp

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cPCR filovirus assay nested PCR

Date: ########
performed by: operator

Goldstein et al, 2018
Target region: L gene: 680nt (1st round) and 630nt (2nd round)

Filo-MOD-FWD	TITTYTCHVTICAAAAICAYTGGG
FiloL.conR	ACCATCATRTTRCTIGGRAAKGCTTT
Filo-MOD-RVS	GCYTCISMIAIIGTTTGIACATT

PCR 1 of 2	1x	unit	1	+5%
H2O		µL		µl
10 x Rxn Buffer		µL		µl
MgCl2		µL		µl
BSA (1 mg/ml)		µL		µl
dUTP's		µL		µl
Filo-MOD-FWD		µL		µl
FiloL.conR		µL		µl
PlatinumTaq		µl		µl
Mix Vol.		µL		
DNA		µL		
Total Vol.		µL		

Temp.	t	X
95 °C	3 min	
92 °C	20 s	*10x TD (-0.5°C ea. Cyc.)
50 °C*	30 s	
72 °C	60 s	
92 °C	20 s	35X
50 °C	30 s	
72 °C	60 s	
72 °C	2 min	
8 °C	~	

expected length: 680 bp

PCR 2 of 2	1x	unit	1	+5%
H2O		µL	µl	µl
10 x Rxn Buffer		µL	µl	µl
MgCl2		µL	µl	µl
dUTP's		µL	µl	µl
Filo-MOD-FWD		µL	µl	µl
Filo-MOD-RVS		µL	µl	µl
PlatinumTaq		µl	µl	µl
Mix Vol.		µL	µl	µl
1st Rd Product		µL		

expected length: 630 bp

Temp.	t	X
95 °C	3 min	
92 °C	20 s	*10 X TD (-0.5°C ea. Cyc.)
50 °C*	30 s	
72 °C	60 s	
92 °C	20 s	40X
50 °C	30 s	
72 °C	60 s	
72°C	2 min	
8 °C	~	

Goldstein, T., et al. (2018). "The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses." Nat Microbiol 3(10): 1084-1089.

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PCR Assays in use for pan-Filoviruses by IPC

cPCR filovirus assay nested PCR

Date: ####### #

performed by: operator

PREDICT SOP, modified from Zhai et al, 2007

Target region: L gene: 680nt (1st round) and 630nt (2nd round)

Filo-MOD-FWD	TITTYTCHVTICAAAAICAYTGGG
FiloL.conR	ACCATCATRTTRCTIGGRAAKGCTTT
Filo-MOD-RVS	GCYTCISMIAIIGTTTGIACATT

PCR 1 of 2	1x	unit	1	5%
H2O		µL		µl
10 x Rxn Buffer		µL		µl
MgCl2	2.5 mM recommended			µl
BSA (1 mg/ml)		µL		µl
dUTP's		µL		µl
Filo-MOD-FWD	0.5 mM recommended			
FiloL.conR	0.5 mM recommended			µl
PlatinumTaq		µl		µl
Mix Vol.		**µL**		
DNA		µL		
Total Vol.		µL		

Temp.	t	X
94 °C	5 min	
94 °C	60 s	40X
52 °C	60 s	
72 °C	60 s	
72 °C	7 min	
8 °C	~	

expected length: 680 bp

PCR 2 of 2	1x	unit	1	5%
H2O		µL	µl	µl
10 x Rxn Buffer		µL	µl	µl
MgCl2	2.5 mM recommended		µl	µl
dUTP's		µL	µl	µl
Filo-MOD-FWD	0.5 mM recommended		µl	µl
Filo-MOD-RVS	0.5 mM recommended		µl	µl
PlatinumTaq		µl	µl	µl
Mix Vol.		µL	µl	µl
1st Rd Product		µL		

Temp.	t	X
94 °C	5 min	
94 °C	60 s	40X
52 °C	60 s	
72 °C	60 s	
72 °C	7 min	
8 °C	~	

expected length: 630 bp

PREDICT SOP, modified from Zhai et al, 2007

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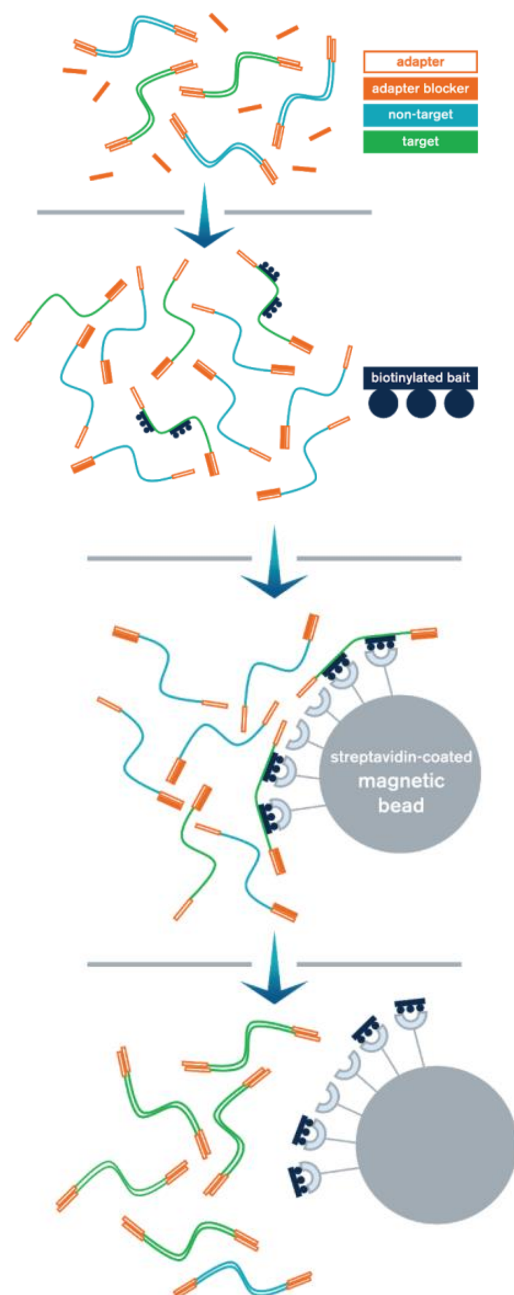
ANNEX 4 : HYBRIDIZATIONCAPTURE_SOP OVERVIEW

INTRODUCTION

myBaits® is an in-solution NGS library target enrichment system, compatible with Illumina®, Ion Torrent®, and essentially any amplifiable sequencing library. We use a versatile nucleic acid synthesis technology to produce biotinylated RNA "baits" that are complementary to your sequence targets. Baits and other reagents for NGS target enrichment are supplied with your myBaits kit. After enrichment with myBaits, libraries may then be sequenced on the aforementioned platforms, or further prepared for PacBio® or Oxford Nanopore Technologies® sequencing.

Procedure Overview

1. Amplified sequencing library, adapter blockers, and other hybridization reagents are combined.
2. Libraries are denatured, allowing blockers to hybridize to adapters. Baits are then introduced and hybridized to targets for several hours.
3. Bait-target hybrids are bound to streptavidin-coated magnetic beads and pulled out of suspension with a magnet.
4. Most non-target DNA is washed away. The remaining library is then amplified and either taken directly to sequencing or further treated.



https://arborbiosci.com/wp-content/uploads/2020/08/myBaits_v5.0_Manual.pdf

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