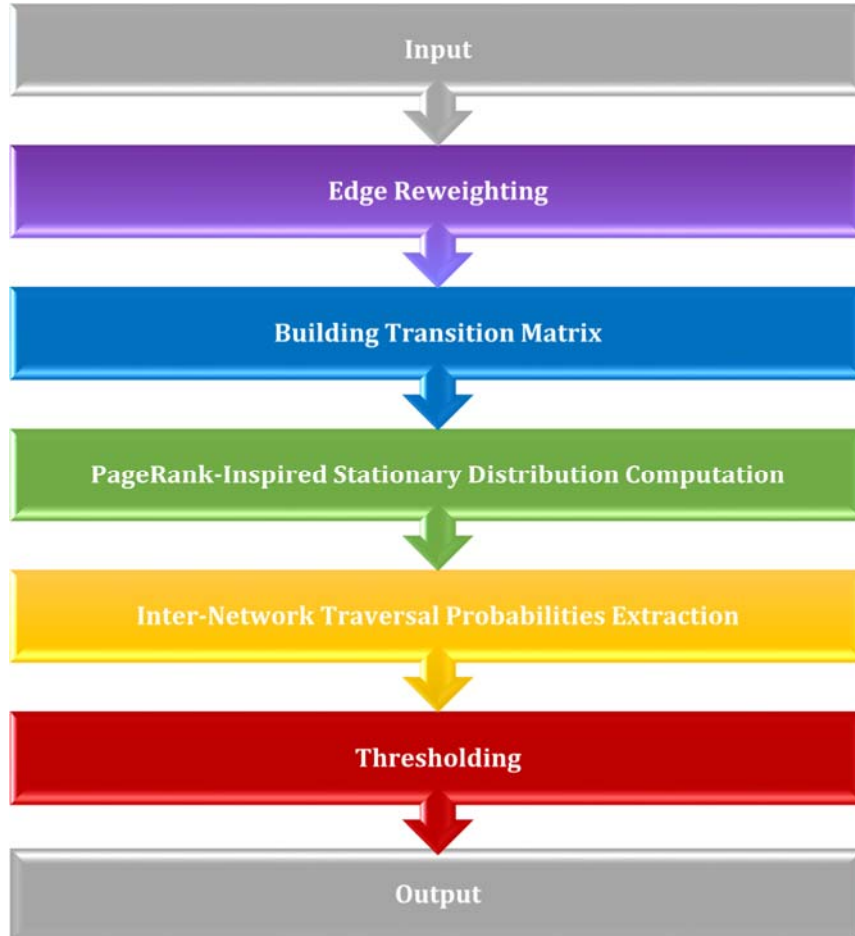


Supplementary Figure 1: PrimAlign processing flow diagram



## Supplementary Text 1: Program input and file formats

The program signature is:

```
PrimAlign.exe networkFile1 networkFile2 internetworkFile outputFile [threshold]
```

Arguments:

networkFile1: path to file with protein-protein interactions defining the 1<sup>st</sup> network  
networkFile2: path to file with protein-protein interactions defining the 2<sup>nd</sup> network  
internetworkFile: path to file with sequence similarities between proteins of both networks  
outputFile: path where to write probable orthologous protein pairs  
threshold: cut-off threshold for candidate orthologs, optional (default value 0.75)

File format: All files are in tab-delimited format with each protein pair on a separate line.

Network files:

Interaction weight is optional. It can be a semantic similarity, for example.

```
ProteinA      ProteinB      [interaction weight A-B]  
ProteinA      ProteinC      [interaction weight A-C]  
ProteinD      ProteinB      [interaction weight D-B]  
...
```

Internetwork file:

Sequence similarity is required, should be either BLAST bit-score or  $-\log(\text{BLAST e-value})$ .  
Proteins from the 1<sup>st</sup> network should be in the 1<sup>st</sup> column.

```
Protein1A     Protein2A     (sequence similarity 1A-2A)  
Protein1B     Protein2C     (sequence similarity 1B-2C)  
Protein1D     Protein2B     (sequence similarity 1D-2B)  
...
```

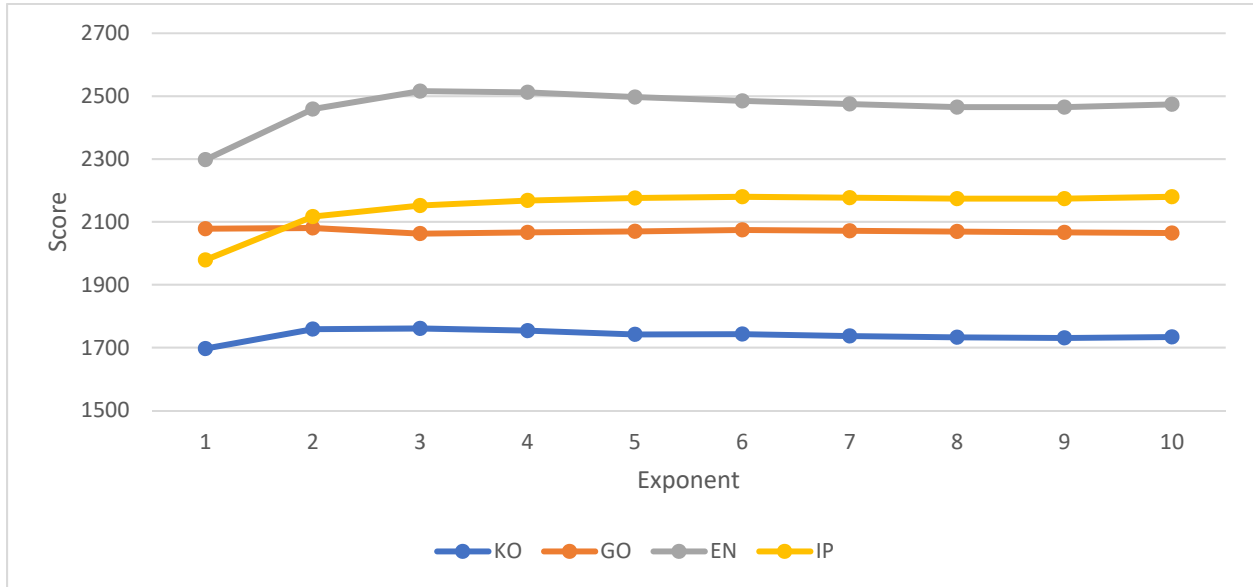
Output file:

Selected ortholog candidates are sorted according to their quality (top pair first).

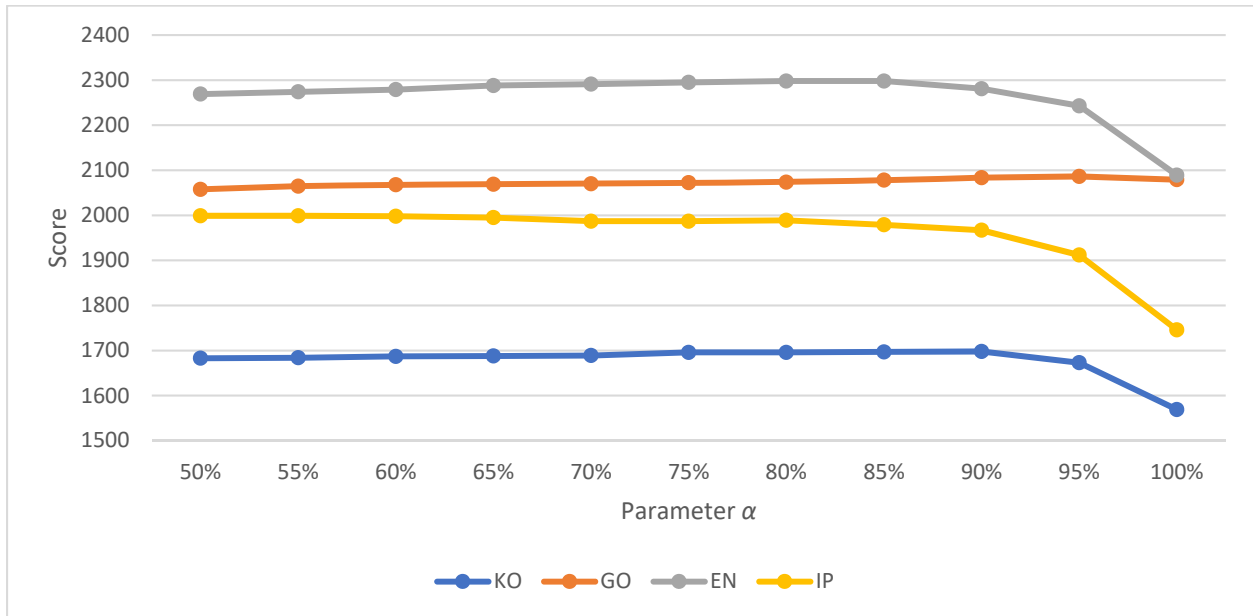
```
Protein1K     Protein2C     (score 1K-2C)  
Protein1I     Protein2M     (score 1I-2M)  
Protein1D     Protein2J     (score 1D-2J)  
...
```

## Supplementary Figures 2: Effect of changing program constants

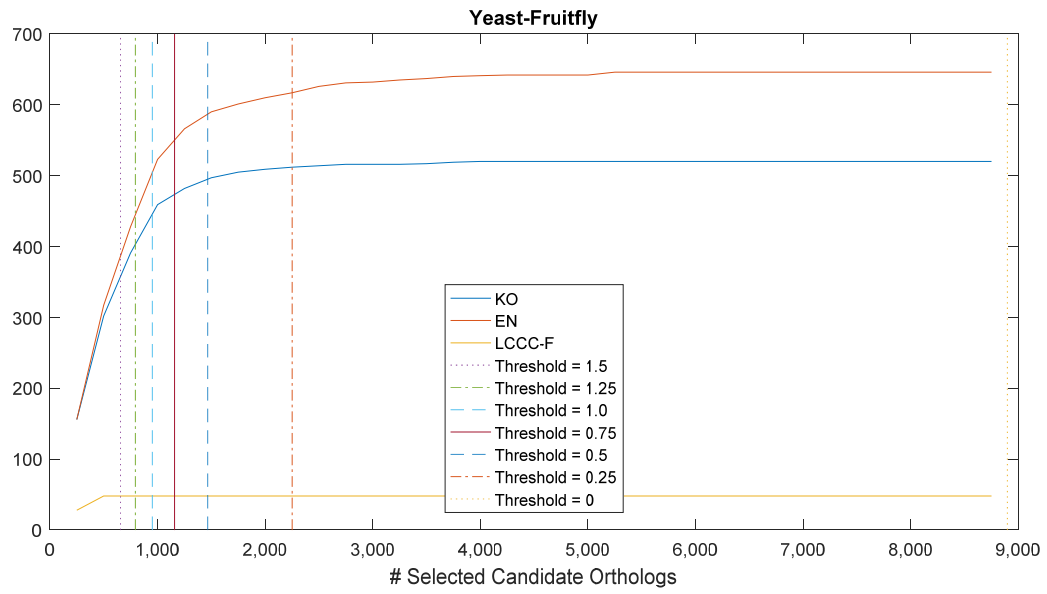
When building the edge reweighting method, various transformations were tried, including linear, logarithmic, and exponential transformations. A positive effect was apparent only for polynomial exponentiation. The results for various exponents and selected evaluation measures are depicted in the figure below. Number 3 was selected as a reasonable exponent.



How different settings of the damping factor affected the results is shown in the figure below. The effect is obvious immediately when  $\alpha < 1$ , as the evaluation measures increase. In concordance with the original PageRank algorithm, the value of 0.85 was selected as an appropriate damping factor.







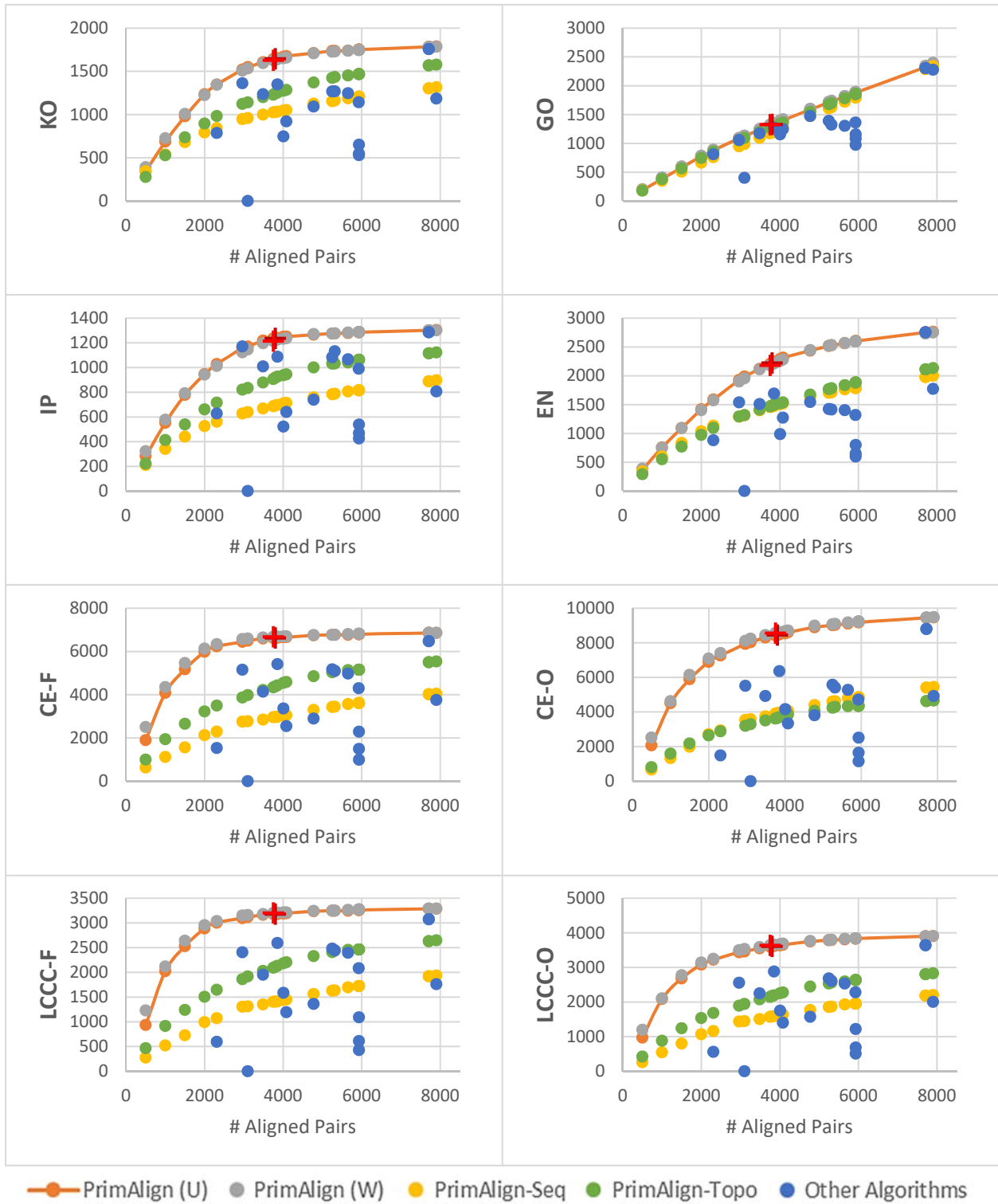
**Supplementary Table 1a: Alignment overview for human-fruit fly alignment**

<b>Aligner</b>	<b>Weighted</b>	<b>AP</b>	<b>Cov</b>	<b>CE</b>	<b>LCCC</b>	<b>cKO</b>	<b>cGO</b>	<b>cEN</b>	<b>cIP</b>
AlignMCL	No	4486	5329	4289	978	4.51	4.22	3.58	6.09
AlignMCL	Yes	4483	5325	4289	978	4.51	4.22	3.58	6.09
AlignNemo	No	890	911	471	90	3.46	3.86	3.27	4.32
AlignNemo	Yes	761	796	456	89	3.28	3.82	3.21	4.09
CUFID	No	1835	3670	1834	805	2.60	4.11	2.24	2.95
CUFID	Yes	1827	3654	1896	793	2.53	3.85	2.19	2.90
HubAlign	No	1124	2248	982	305	16.53	6.44	15.61	17.84
IsoRankN	No	0	0	0	0	NA	NA	NA	NA
MAGNA++	No	2120	4240	764	204	5.06	5.45	4.39	6.22
MI-GRAAL	No	NA	NA	NA	NA	NA	NA	NA	NA
NETAL	No	177	354	2	1	$\infty$	7.58	$\infty$	$\infty$
NetCoffee	No	282	564	140	18	2.12	3.08	2.14	2.45
NetworkBLAST	No	633	652	235	32	4.83	4.02	4.55	5.60
NetworkBLAST	Yes	105	145	16	3	4.77	3.93	4.57	5.83
PINALOG	No	945	1890	978	305	3.99	4.67	3.95	4.33
PrimAlign	No	2761	3342	4289	1097	2.58	3.67	2.10	3.62
PrimAlign	Yes	2762	3325	4371	1080	2.56	3.65	2.11	3.63
SANA	No	2120	4240	1118	251	5.65	5.35	4.24	6.95
SMETANA	No	2254	3295	2409	780	2.79	3.73	2.27	3.52
SMETANA	Yes	2298	3378	2364	780	2.68	3.74	2.24	3.46
WAVE	No	2120	4240	1734	672	3.67	4.46	2.99	4.14

**Supplementary Table 1b: Alignment overview for yeast-fruit fly alignment**

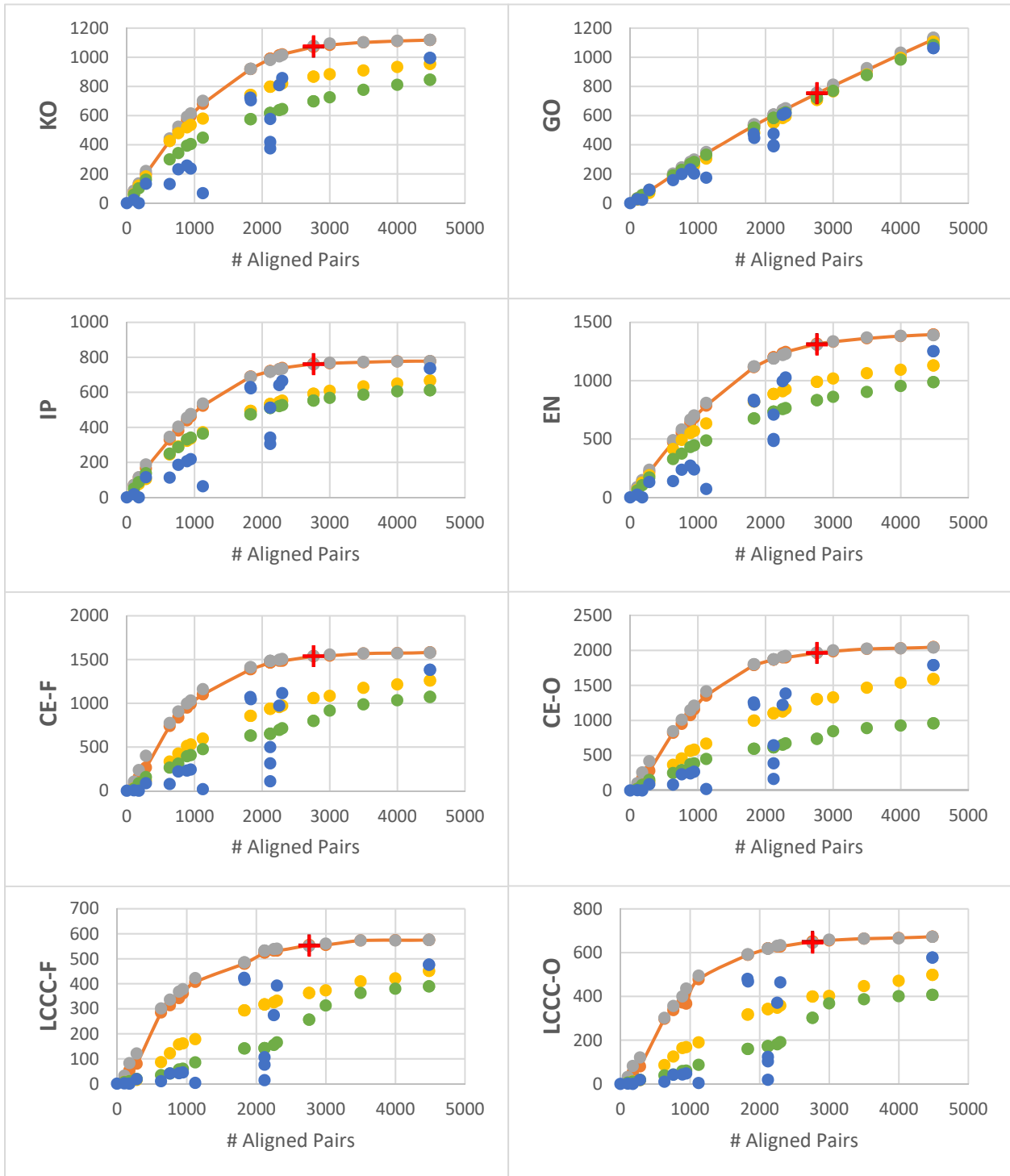
<b>Aligner</b>	<b>Weighted</b>	<b>AP</b>	<b>Cov</b>	<b>CE</b>	<b>LCCC</b>	<b>cKO</b>	<b>cGO</b>	<b>cEN</b>	<b>cIP</b>
AlignMCL	4486	1474	2006	928	139	3.43	3.20	3.00	4.22
AlignMCL	4483	1474	2006	928	139	3.43	3.20	3.00	4.22
AlignNemo	890	352	383	175	32	2.77	3.08	2.86	3.56
AlignNemo	761	314	338	181	32	3.11	3.05	3.11	3.61
CUFID	1835	1223	2446	674	77	3.18	3.53	2.86	3.56
CUFID	1827	1306	2612	1144	192	3.22	3.30	2.95	3.61
HubAlign	1124	1124	2248	1410	231	2.28	2.74	2.43	2.96
IsoRankN	0	0	0	0	0	NaN	NaN	NaN	NaN
MAGNA++	2120	2120	4240	870	171	6.09	4.82	5.44	7.07
MI-GRAAL	No	NA	NA	NA	NA	NA	NA	NA	NA
NETAL	No	386	772	20	2	$\infty$	6.50	$\infty$	$\infty$
NetCoffee	No	237	474	114	16	2.12	2.54	2.39	2.58
NetworkBLAST	No	241	272	67	8	4.55	3.50	4.46	5.13
NetworkBLAST	Yes	0	0	0	0	NaN	NaN	NaN	NaN
PINALOG	No	928	1856	1070	209	3.02	3.24	3.01	3.58
PrimAlign	No	1150	1547	919	275	2.41	2.94	2.09	3.08
PrimAlign	Yes	1159	1532	932	282	2.45	2.93	2.10	3.12
SANA	No	2119	4238	1344	199	3.89	3.91	4.13	5.06
SMETANA	No	1124	1763	764	102	2.80	3.06	2.52	3.43
SMETANA	Yes	1173	1794	759	99	2.93	3.08	2.57	3.54
WAVE	No	2120	4240	1680	525	4.22	4.00	3.96	5.11

Supplementary Figure 4a: Results of human-yeast alignment



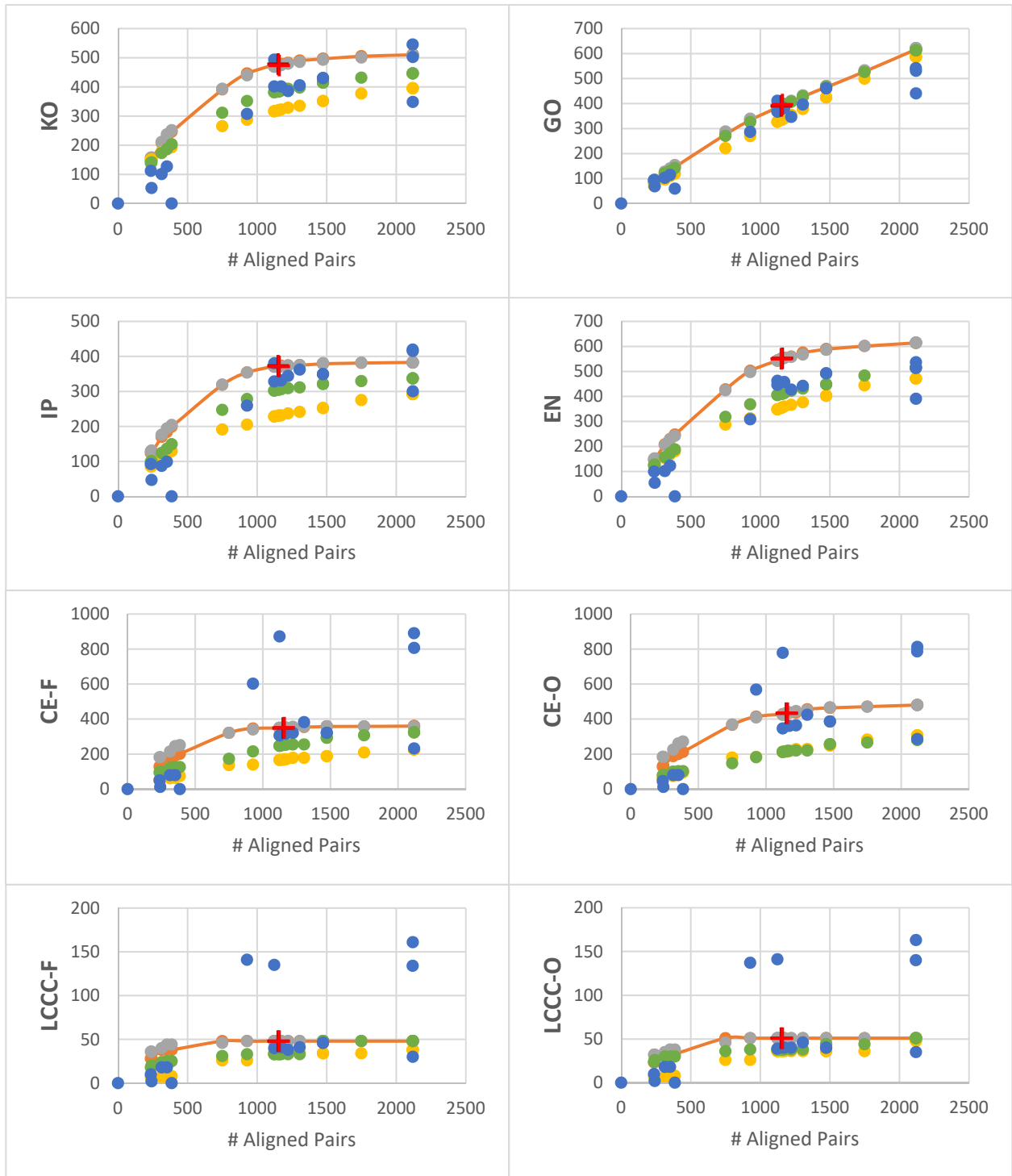


Supplementary Figure 4b: Results of human-fly alignment



—●— PrimAlign (U)  
 ● PrimAlign (W)  
 ● PrimAlign-Seq  
 ● PrimAlign-Topo  
 ● Other Algorithms

Supplementary Figure 4c: Results of yeast-fruit fly Alignment



—●— PrimAlign (U)   ● PrimAlign (W)   ● PrimAlign-Seq   ● PrimAlign-Topo   ● Other Algorithms



**Supplementary Table 2b: Statistical comparison of PrimAlign with others in yeast-fruit fly alignment**

Algorithm	Weighted	KO	GO	EN	IP	CE-F	CE-H	LCCC-F	LCCC-H
AlignMLC	N	**		***			**		
AlignMLC	Y	**		***			**		
AlignNemo	N	***	**	***	***	***	***	**	*
AlignNemo	Y	***	**	***	***	***	***	**	*
CUFID	N	***	***	***			**		
CUFID	Y	***	**	***					
HubAlign	N		*	***		***	***	***	***
IsoRankN	N	NA	NA	NA	NA	NA	NA	NA	NA
MAGNA++	N	***	***	***	***	***	***	*	
MI-GRAAL	N	NA	NA	NA	NA	NA	NA	NA	NA
NETAL	N	***	***	***	***	***	***	***	***
NetCoffee	N	**		***	**	***	***	**	*
NetworkBLAST	N	***	***	***	***	***	***	***	***
NetworkBLAST	Y	NA	NA	NA	NA	NA	NA	NA	NA
PINALOG	N	***	***	***	***	***	***	***	***
SANA	N		***	***		***	***	***	***
SMETANA	N	**		***			**		
SMETANA	Y	**		***	*		**		
WAVE	N		***	**		***	***	***	***

**Supplementary Table 3: Running time**

Algorithm	Weighted	RunTime [s]			RunTime Ratio	
		h-y	h-f	y-f	h-y / h-f	h-f / y-f
NetCoffee	N	4	3	2	1.3	1.5
PrimAlign	Y	21	12	2	1.8	6.0
PrimAlign	N	22	13	2	1.7	6.5
CUFID	N	321	259	38	1.2	6.8
SMETANA	N	337	275	41	1.2	6.7
CUFID	Y	377	310	45	1.2	6.9
SMETANA	Y	382	320	47	1.2	6.8
AlignMCL	N	558	73	11	7.6	6.6
AlignMCL	Y	569	68	11	8.4	6.2
WAVE	N	666	68	30	9.8	2.3
HubAlign	N	721	31	12	23.3	2.6
SANA	N	942	632	401	1.5	1.6
AlignNemo	N	1454	27	5	53.9	5.4
AlignNemo	Y	1848	27	5	68.4	5.4
NETAL	N	2649	246	74	10.8	3.3
PINALOG	N	17346	31674	2261	0.5	14.0
MAGNA++	N	39131	28833	8883	1.4	3.2
NetworkBLAST	Y	122217	418	57	292.4	7.3
NetworkBLAST	N	125480	509	70	246.5	7.3
IsoRankN	N	348070	11546	3813	30.1	3.0
MI-GRAAL	N	N/A	N/A	est >1198379	N/A	N/A