I2BC
Institut de Biologie Intégrative de la Cellule

Characterization of the Radiation Desiccation Response regulon of the radioresistant bacterium *Deinococcus radiodurans* by integrative genomic analyses

cea
cnrs
Université Paris Sud
université Paris-Saclay
université Paris-Saclay

The Deinococcaceae

Deinococcus radiodurans

D. deserti

D. gobiensis

Irradiated meat can (1956)

D. geothermalis

D. radiophilus

Several records

- **Resistance to ionizing radiation**

  No loss of viability when exposed to a dose of 6000 Gy

  (~200 DSB, 3000 SSB, >1000 damaged bases in the genome)

- **Resistance to UV**

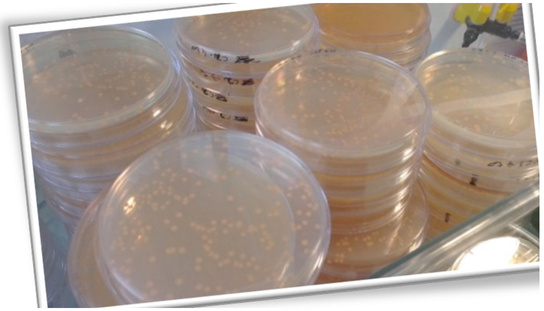
  No loss of viability when exposed to a dose of 500 J/m^2

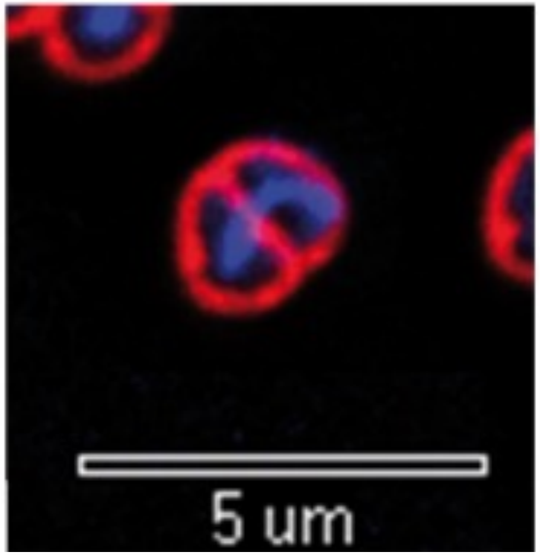
  (5000 pyrimidine dimers per genome)

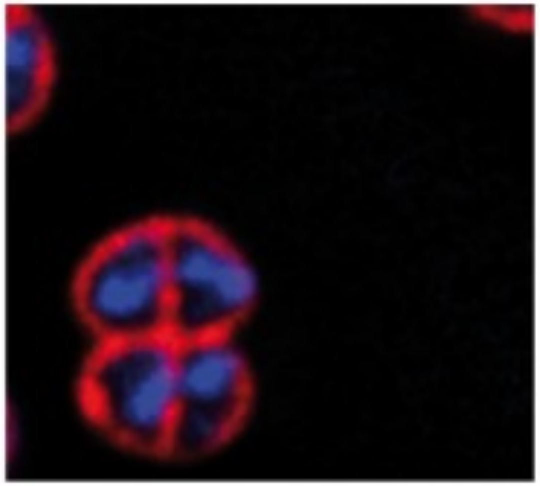
- **Resistance to the desiccation**

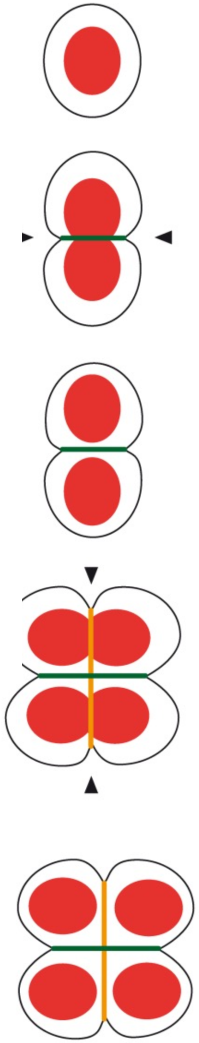
  85% viability after 2 years in an < 5 % wet environment

Deinococcus radiodurans









Monocoque

Premier plan de division

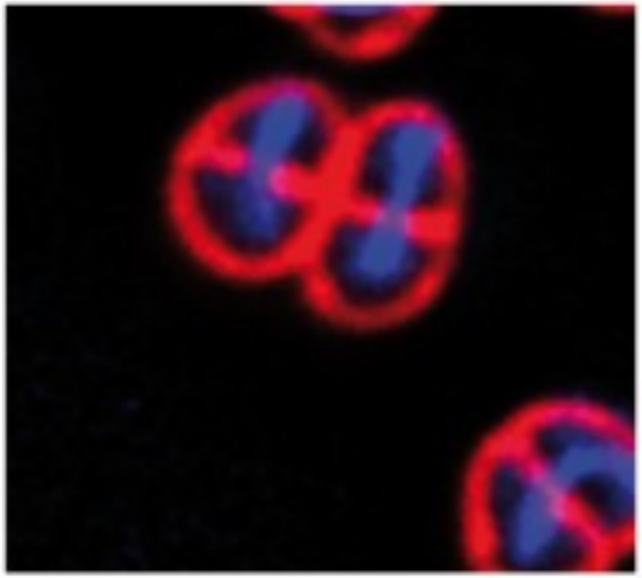
first division plan

Diplocoque
Les nucléoïdes sont sé
mais les cellules ne sont pa

second division

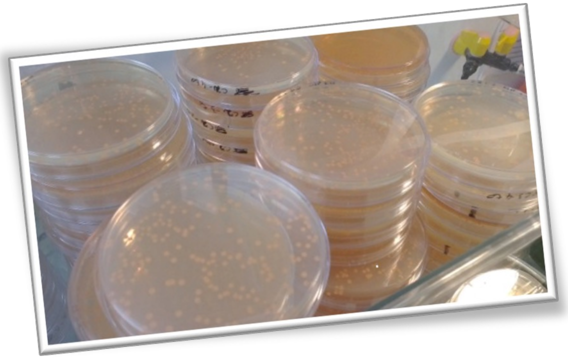
Deuxième plan de division perpend
à celui de la première divisio

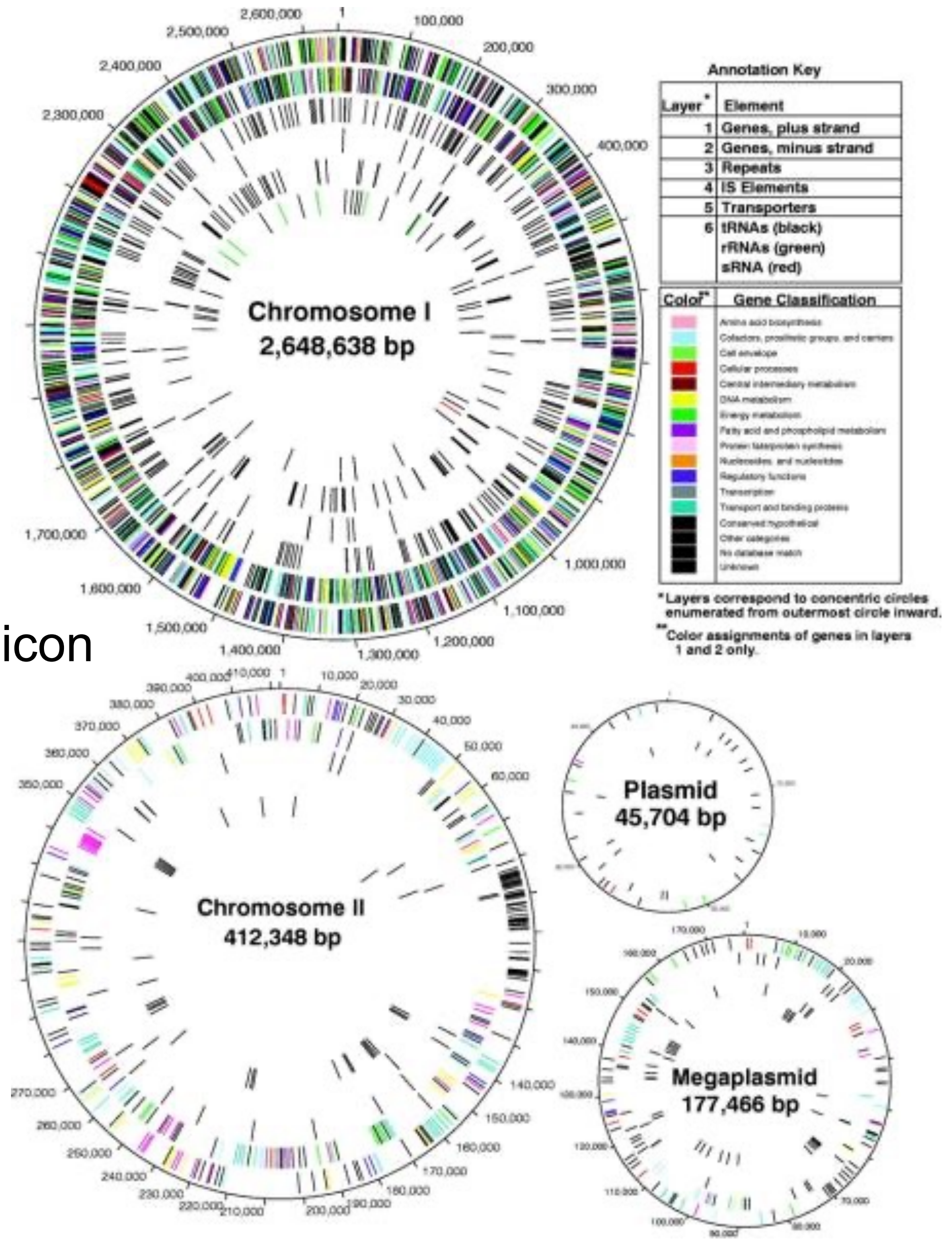
Tétracoque
Les nucléoïdes issus de la deuxième
sont ségrégés mais les cellules restent



Membrane : FM4-64 Dye
DNA: DAPI

Deinococcus radiodurans





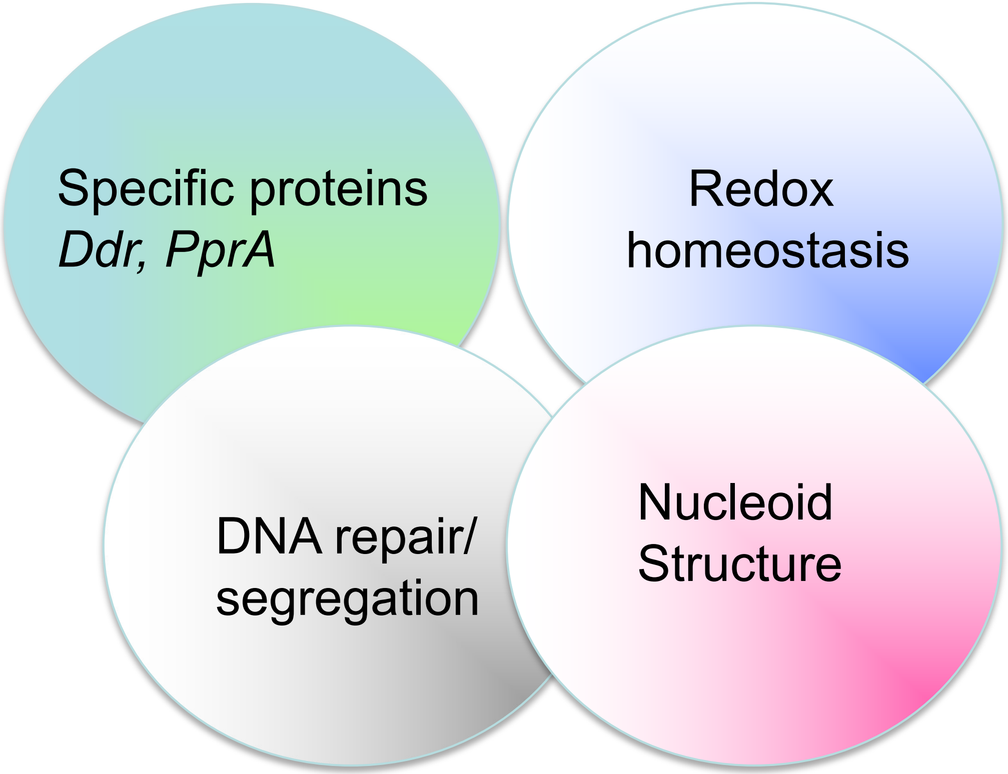
White et al,1999

4-8 (10) copies of each replicon

Radioresistance pathways:

Deinococcus radiodurans

Multiscale approaches : Genetic, Molecular Biology, Transcriptome, Proteome (LC-MS/ICP-MS), Microscopy, Structural Biology, BioInformatics, Physiology.



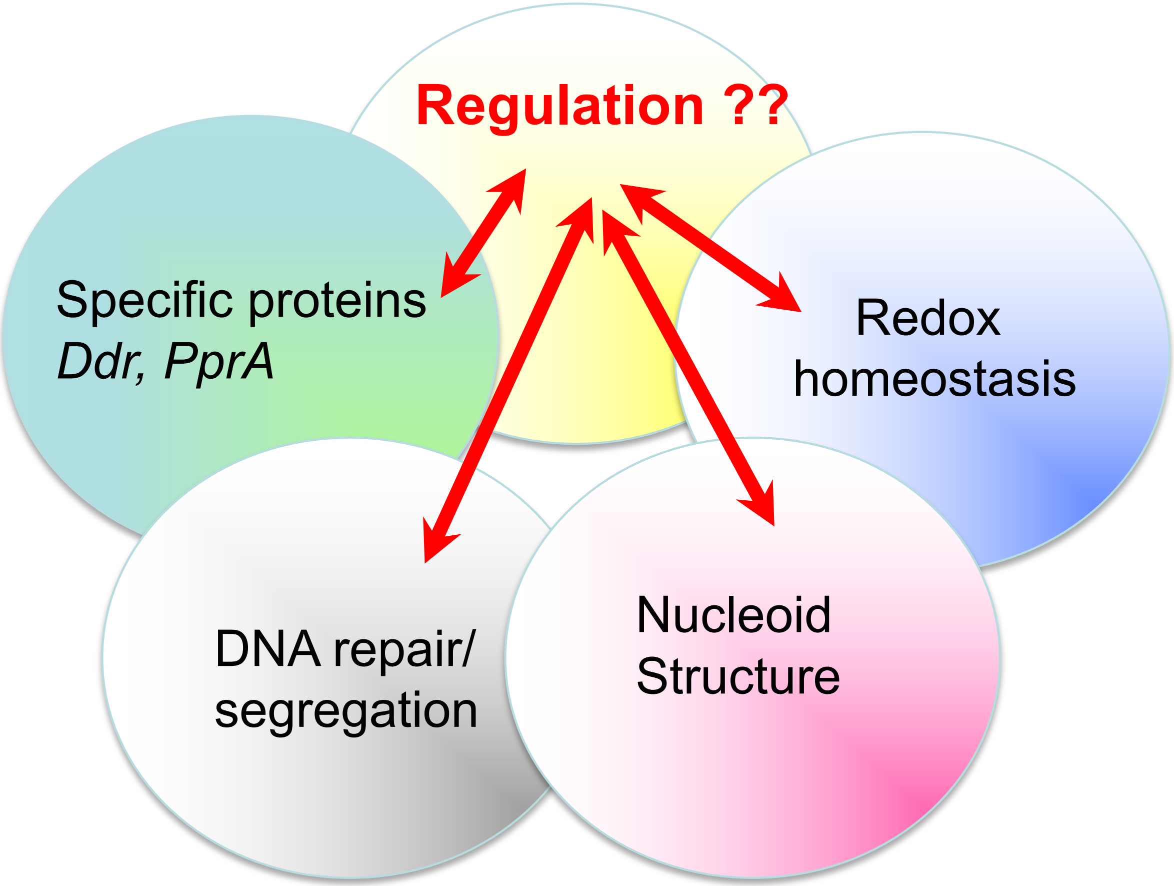
Specific proteins
Ddr, PprA

Redox homeostasis

DNA repair/ segregation

Nucleoid Structure

Regulation of the different pathways ?



Regulation ??

Specific proteins
Ddr, PprA

Redox
homeostasis

DNA repair/
segregation

Nucleoid
Structure

In many bacteria as *Escherichia coli*

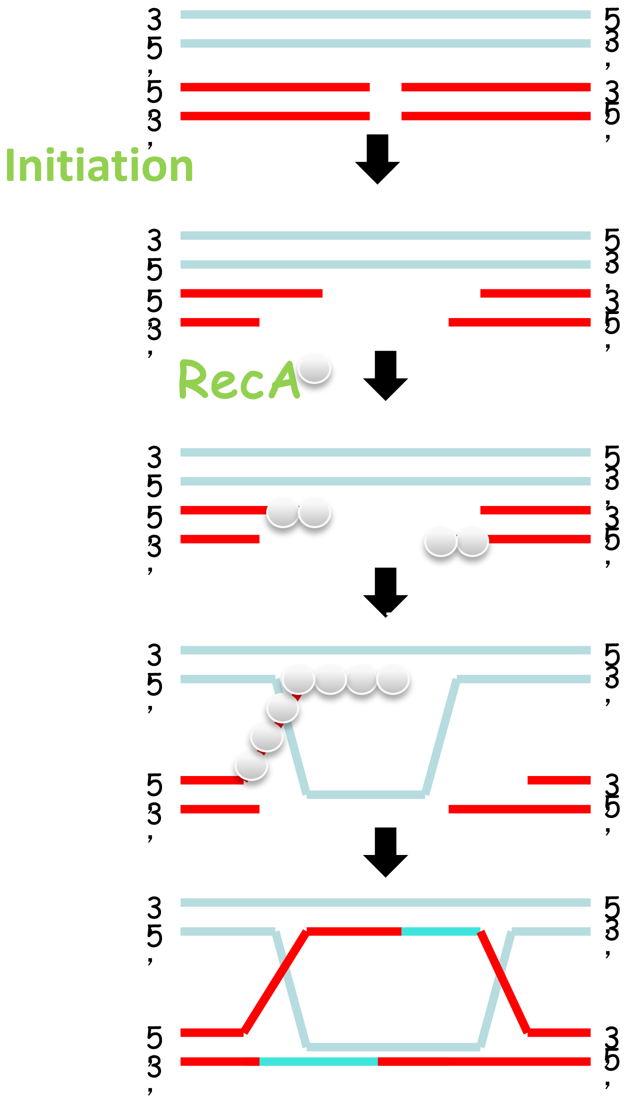
Many bacteria use the RecA/LexA-controlled SOS response to induce DNA repair genes

RecA* stimulates the autocleavage of LexA, the repressor of the SOS regulon, then the DNA repair genes are expressed

***Deinococcus radiodurans* encodes two *lexA* genes but induction of *recA* and other DNA repair genes occurs in an SOS-independent manner**

Genome annotation → >100 putative regulators

recA filaments invade intact duplex



search for homologous regions

D-loop formation

In many bacteria as *Escherichia coli*

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RecA* stimulates the autocleavage of LexA, the repressor of the SOS regulon, then the DNA repair genes are expressed

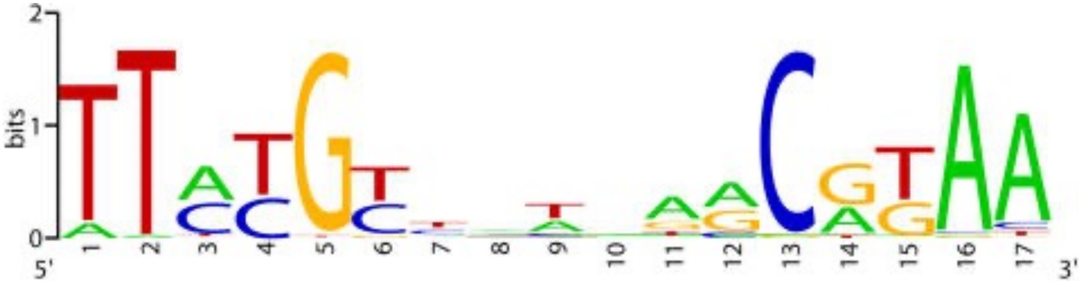
***Deinococcus radiodurans* encodes two *lexA* genes but induction of *recA* and other DNA repair genes occurs in an SOS-independent manner**

Genome annotation → >100 putative regulators

Tanaka et al (2004): After exposition to gamma rays : 70 genes are rapidly and strongly upregulated (ddrO):

Ddr: DNA damage response

Analysis of the promotor sequences of these genes including ddrO



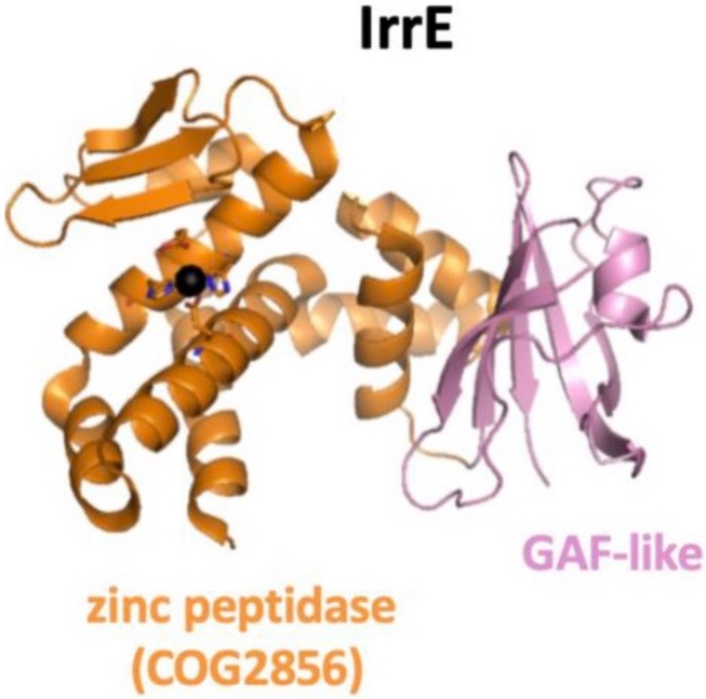
Makarova et al 2007

Radiation/Desiccation Response Motif (RDRM)

In *Deinococcus* species 14-24 genes under the control of ddrO

A second regulator ? the IrrE protein (PprI)

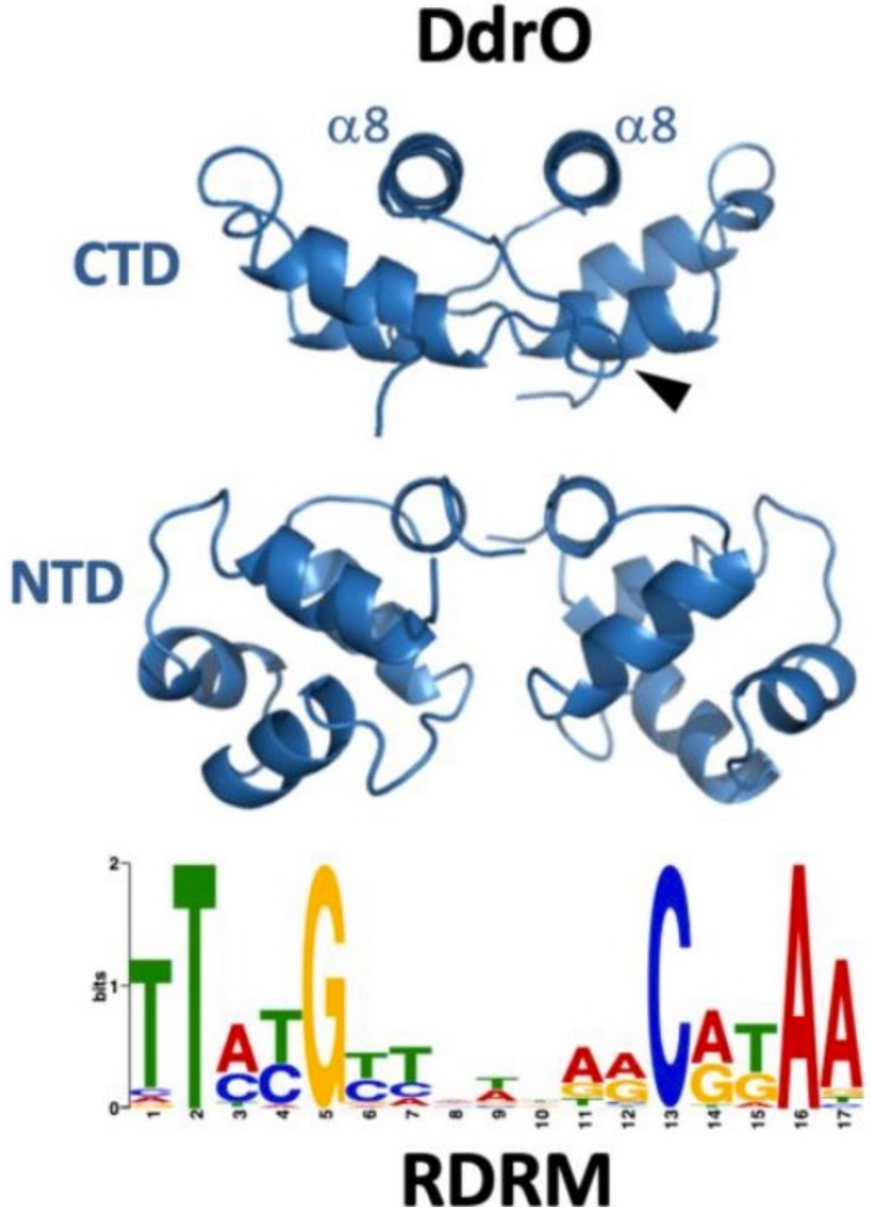
Radiation-induced expression of *recA* and several other genes was abolished in Δ*irrE* mutants, indicating that IrrE could be a positive regulator of RDR genes



Vujicic-Zagar, A et al 2009

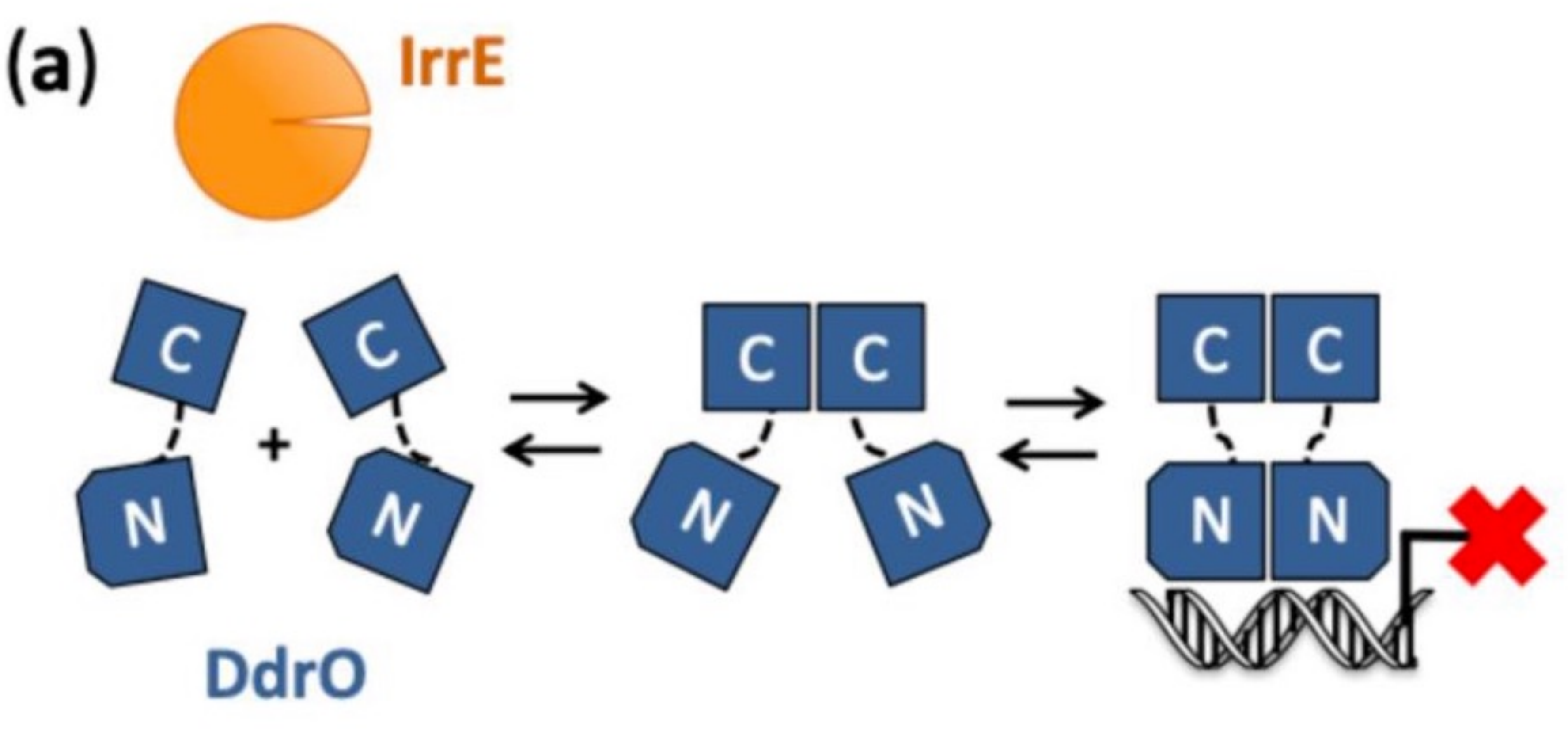
The DrdO transcriptional regulator

AGENCE NATIONALE DE LA RECHERCHE
ANR
NOVOREP



DdrO Structure: de Groot et al, NAR 2019

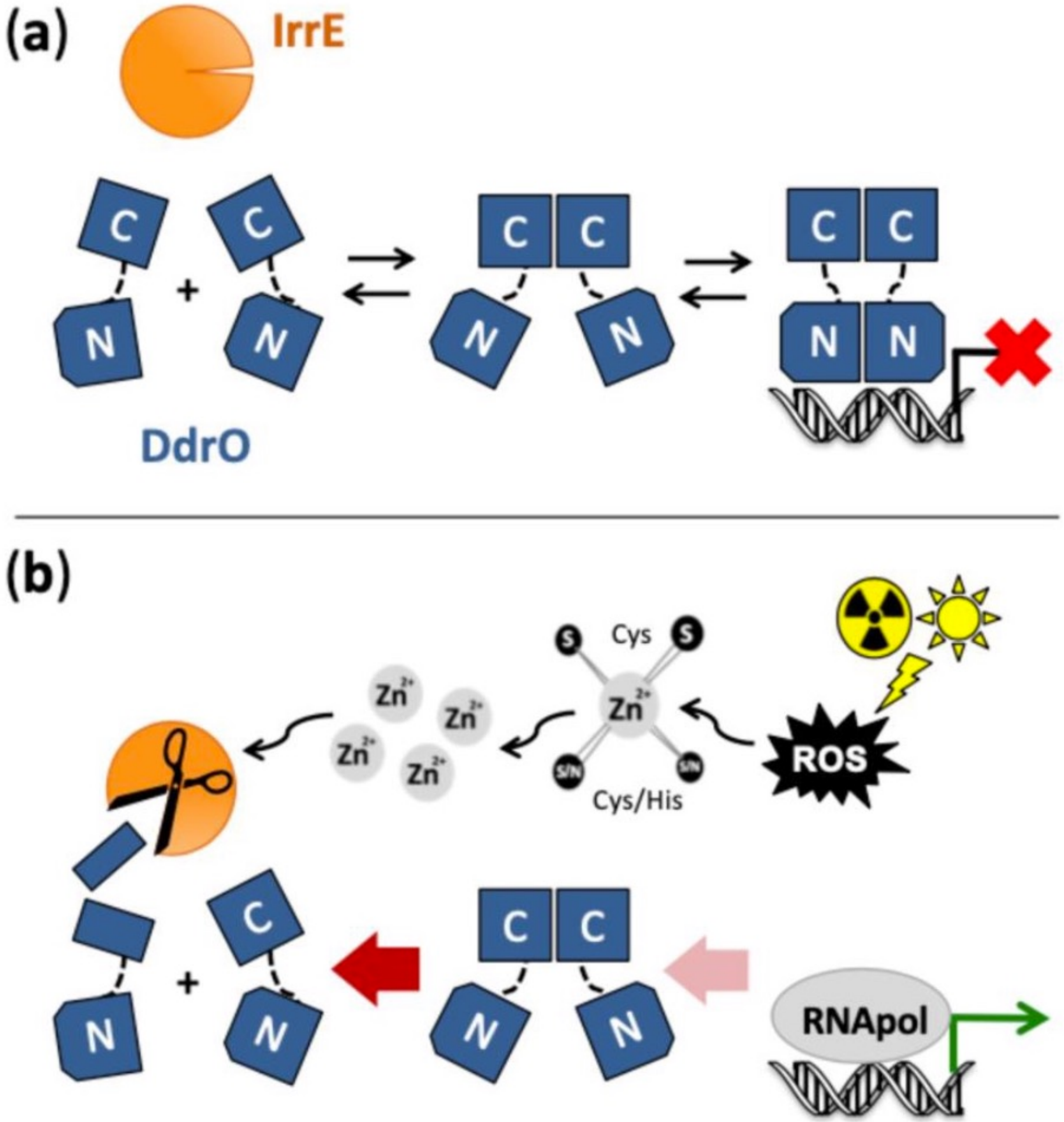
The DrdO/IrrE transcriptional regulators



A. De groot and L. Blanchard, 2021

The DrdO/IrrE transcriptional regulators

ANR NOVOREP



A. De groot and L. Blanchard, 2021

How long does it take to repair damage?

About 2-3H after exposition to a dose of 6 kGy

How this system is locked again ?

When damage are repaired, the Zinc-binding proteins trap again the metal, the concentration of free ions decreases and the IrrE protease is no longer activated. The DdrO proteins are capable of binding to promoters of target genes and repress their expression. The system is locked again.

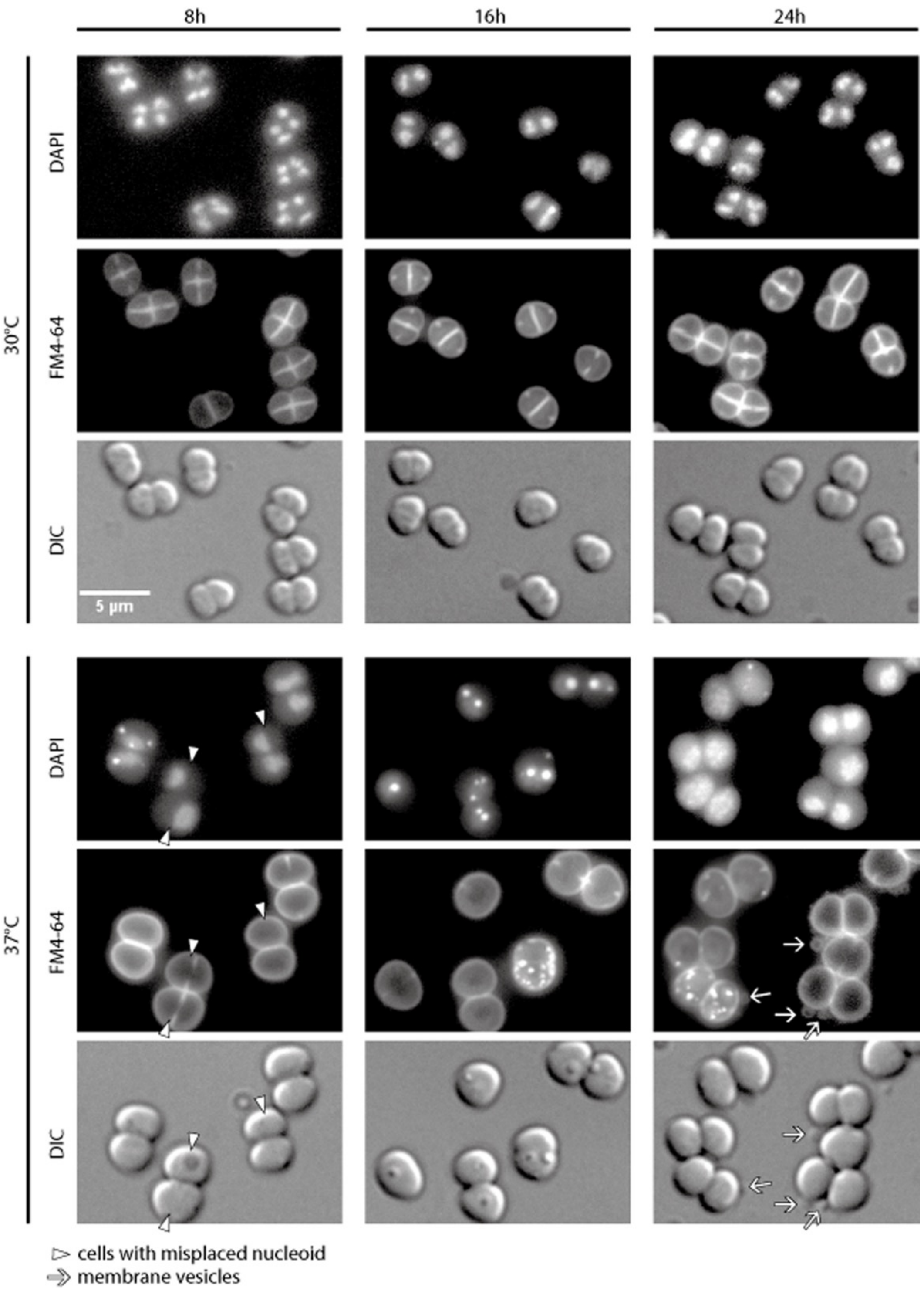
What happens if genes that are under the control of ddrO are constitutively expressed?

↓

Cells die!!

In contrast to *irrE*, *ddrO* is an essentiel gene

Devigne et al, 2015



(Devigne *et al*, 2015)

Control
Normal

APOPTOSE-LIKE

DNA fragmentation
Defects in cell division

membrane blebbing

What happens if genes that are under the control of ddrO are constitutively expressed?

↓

Cells die!!

In contrast to *irrE*, *ddrO* is an essentiel gene

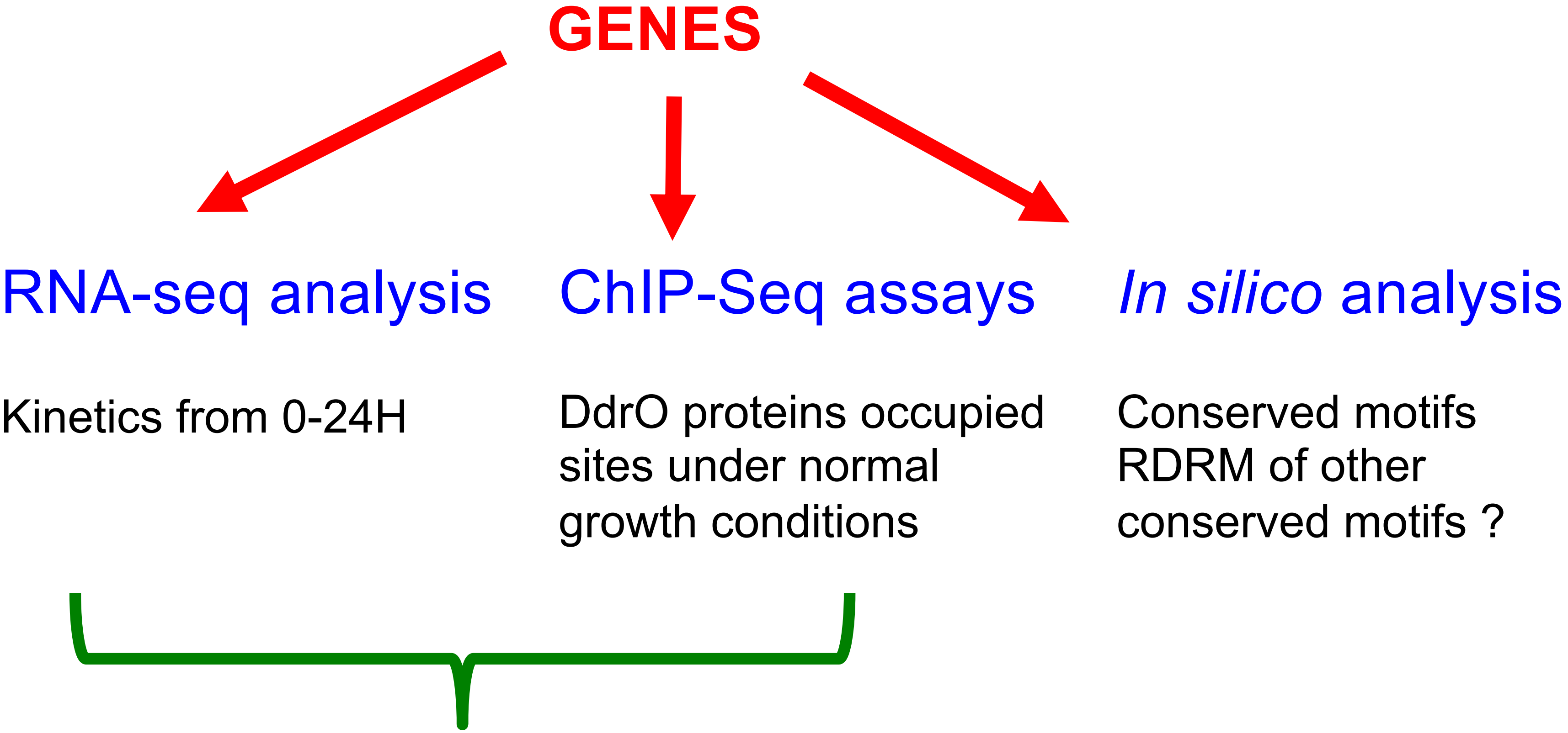
↓

How to characterize the *ddO* regulon ?

In *Deinococcus* species 14-24 genes under the control of ddrO

ddrO regulon

To date, the DdO regulon is poorly characterized.



GENES

RNA-seq analysis

Kinetics from 0-24H

ChIP-Seq assays

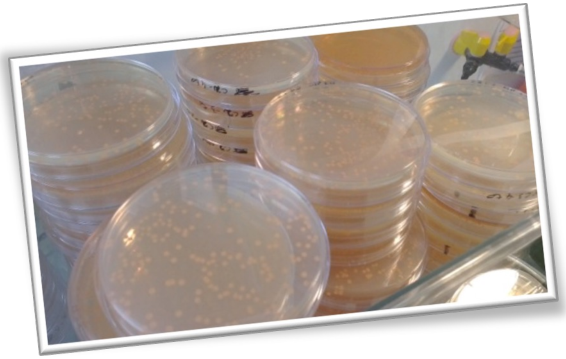
DdrO proteins occupied sites under normal growth conditions

In silico analysis

Conserved motifs RDRM of other conserved motifs ?

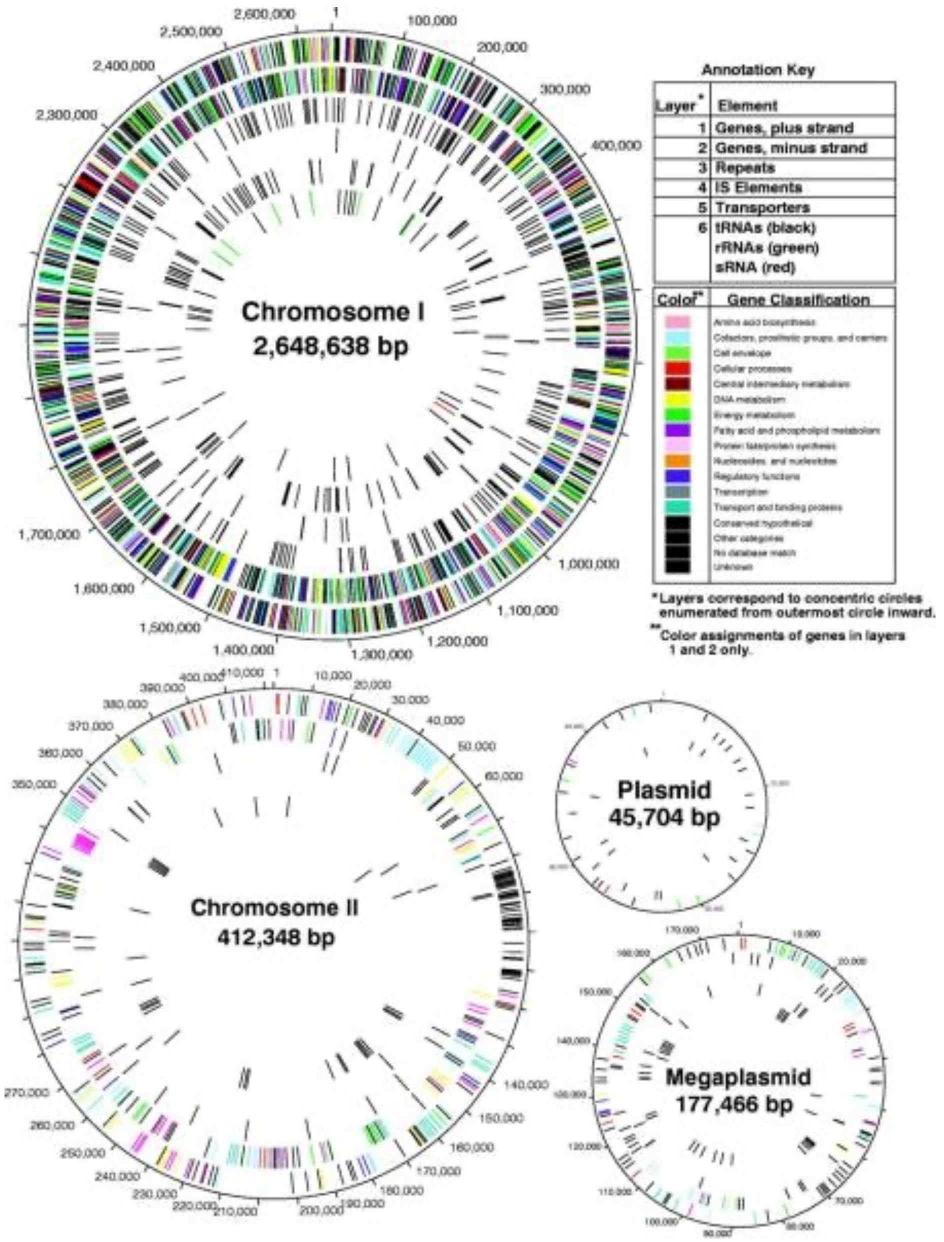
I2BC High throughput sequencing platform : we resequenced the *D. radiodurans* genome

Deinococcus radiodurans genome



White et al,1999

Hua and Hua, 2016



Layer*	Element
1	Genes, plus strand
2	Genes, minus strand
3	Repeats
4	IS Elements
5	Transporters
6	tRNAs (black) rRNAs (green) sRNA (red)

Color**	Gene Classification
■	Amino acid biosynthesis
■	Cofactors, prosthetic groups, and carriers
■	Cell envelope
■	Cellular processes
■	Central intermediary metabolism
■	DNA metabolism
■	Energy metabolism
■	Fatty acid and phospholipid metabolism
■	Protein fate/protein synthesis
■	Nucleosides, and nucleotides
■	Regulatory functions
■	Transcription
■	Transport and binding proteins
■	Conserved hypothetical
■	Other categories
■	No database match
■	Unknown

*Layers correspond to concentric circles enumerated from outermost circle inward.

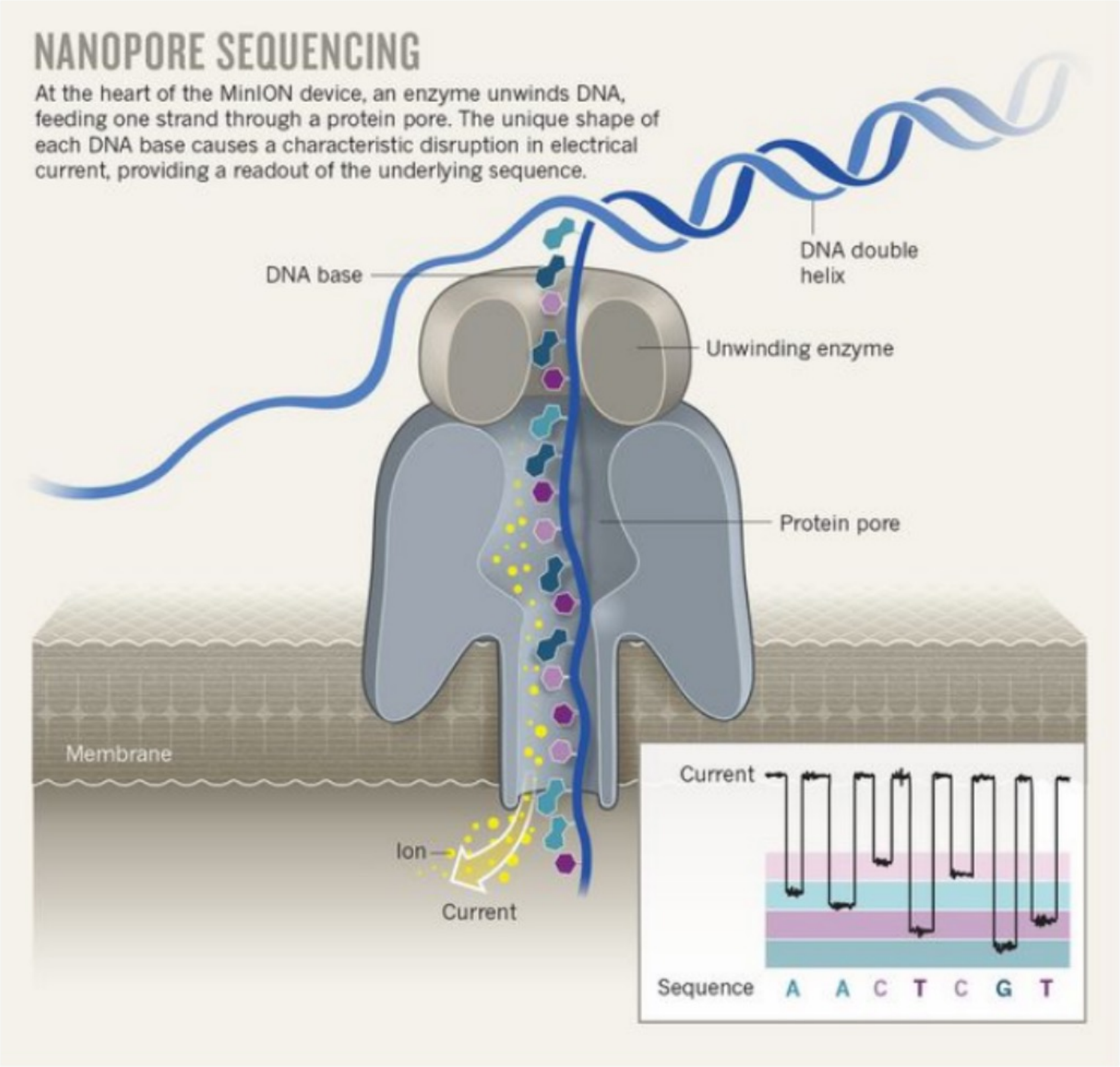
**Color assignments of genes in layers 1 and 2 only.

	Replicon	ID	Percentage of pairs of orthologs*	CDS	Total CDS	nt.	GC %
Hua & Hua (2016)	Chr 1	CP015081	96,43	2523	3079	2646742	67,07
	Chr 2	CP015082	96,02	352		433133	66,77
	Megaplasmid	CP015083	81,05	153		203183	62,98
	Plasmid	CP015084	62,75	51		61707	56,55
White et al (1999)	Chr 1	DRA1	84,37	2629	3181	2648638	67,01
	Chr 2	DRA2	85,05	368		412348	66,69
	Megaplasmid	DRA3	77,24	145		177466	63,19
	Plasmid	DRA4	61,54	39		45704	56,15
This work	Chr 1	DRO	100	2594	3147	2644251	67,08
	Chr 2	DRO_A	100	364		412138	66,65
	Megaplasmid	DRO_B	100	148		177322	63,21
	Plasmid	DRO_C	100	41		45508	56,26

Illumina sequencing coupled to Nanopore sequencing:
3578820 nt (450X coverage)

Cours C. Fairhead

Nanopore sequencing
Oxford Nanopore Technologies



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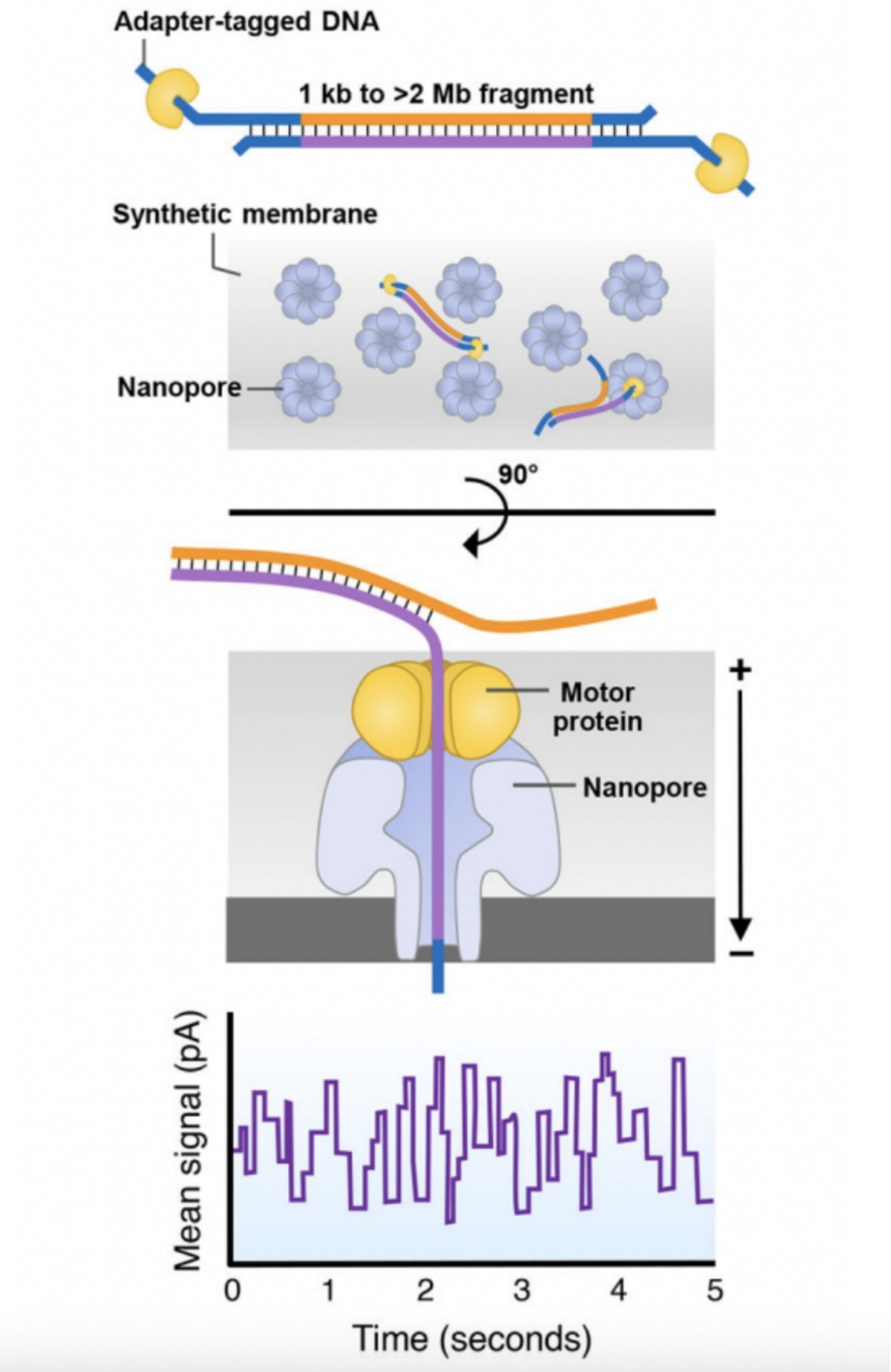

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* Percentage of pairs of orthologs (at a threshold of 90 %max bit score)

Méthodes de séquençage: Nanopore + Illumina

ddrO regulon

NOVOREP

To date, the DdO regulon is poorly characterized.

GENES

RNA-seq analysis

Kinetics from 0-24H

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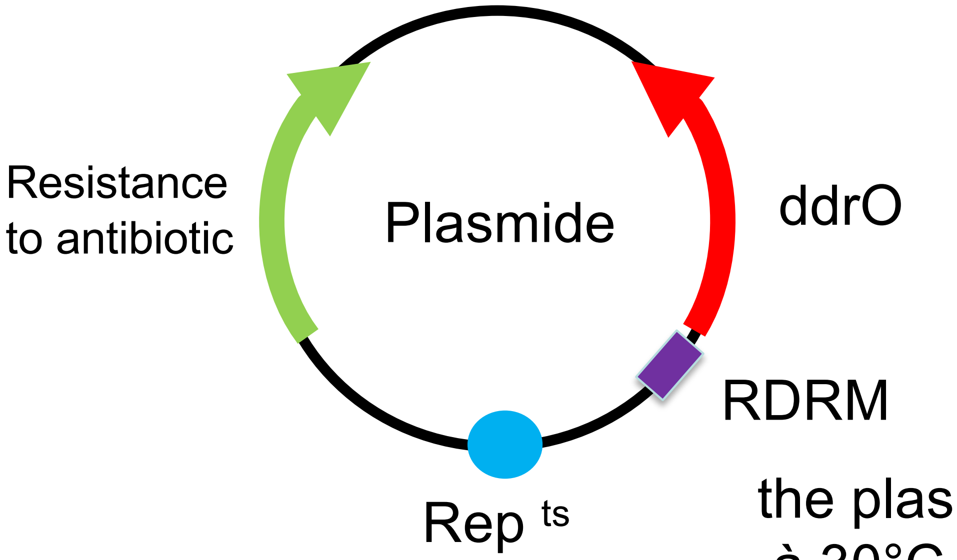
1-RNA-seq analysis

Comparison of transcriptomic profiles of cells expressing or not DdrO.

But ddrO is an essential gene.

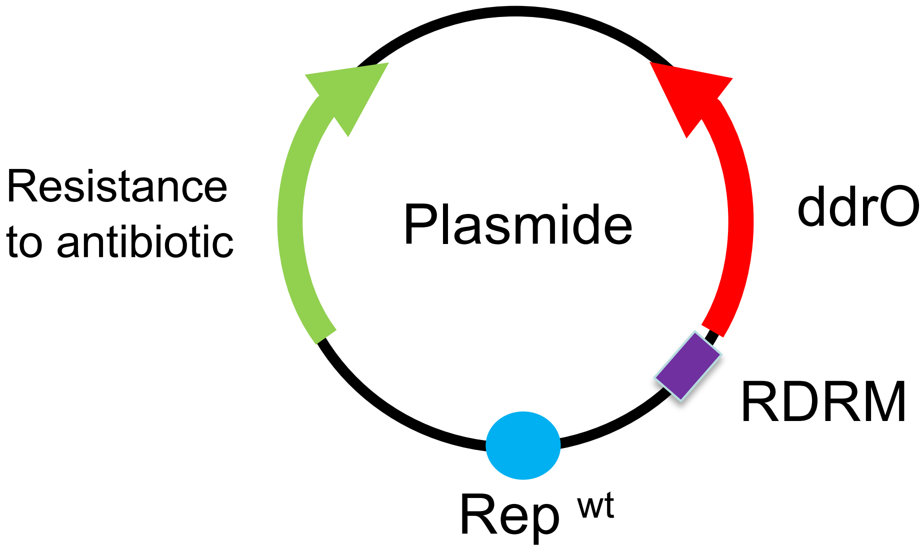
Conditional expression system

Devigne et al, 2015



The Rep (DnaA-like) protein used to initiate the replication of the plasmid

the plasmid is able to replicate at à 30°C but not at 37°C



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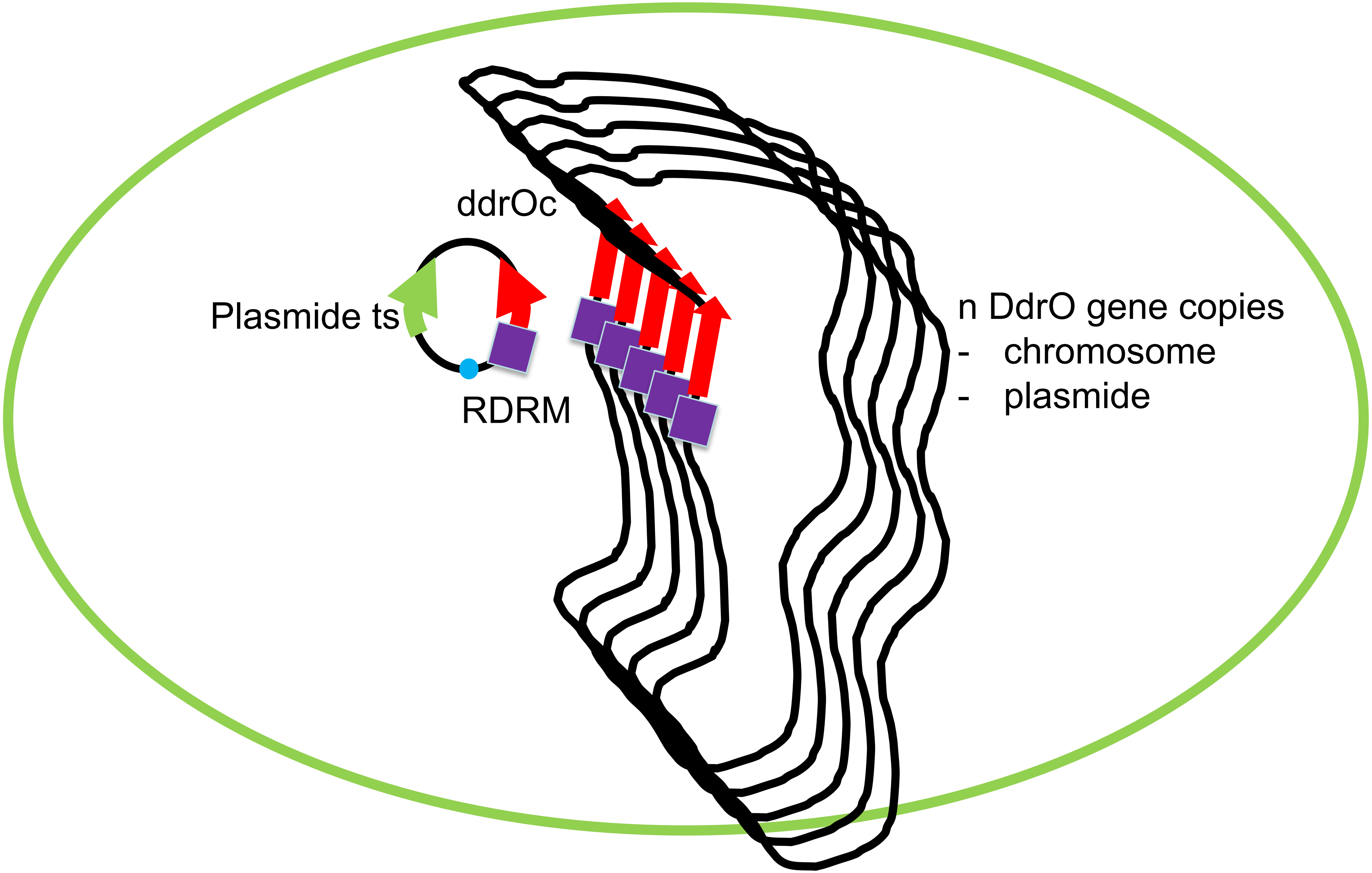
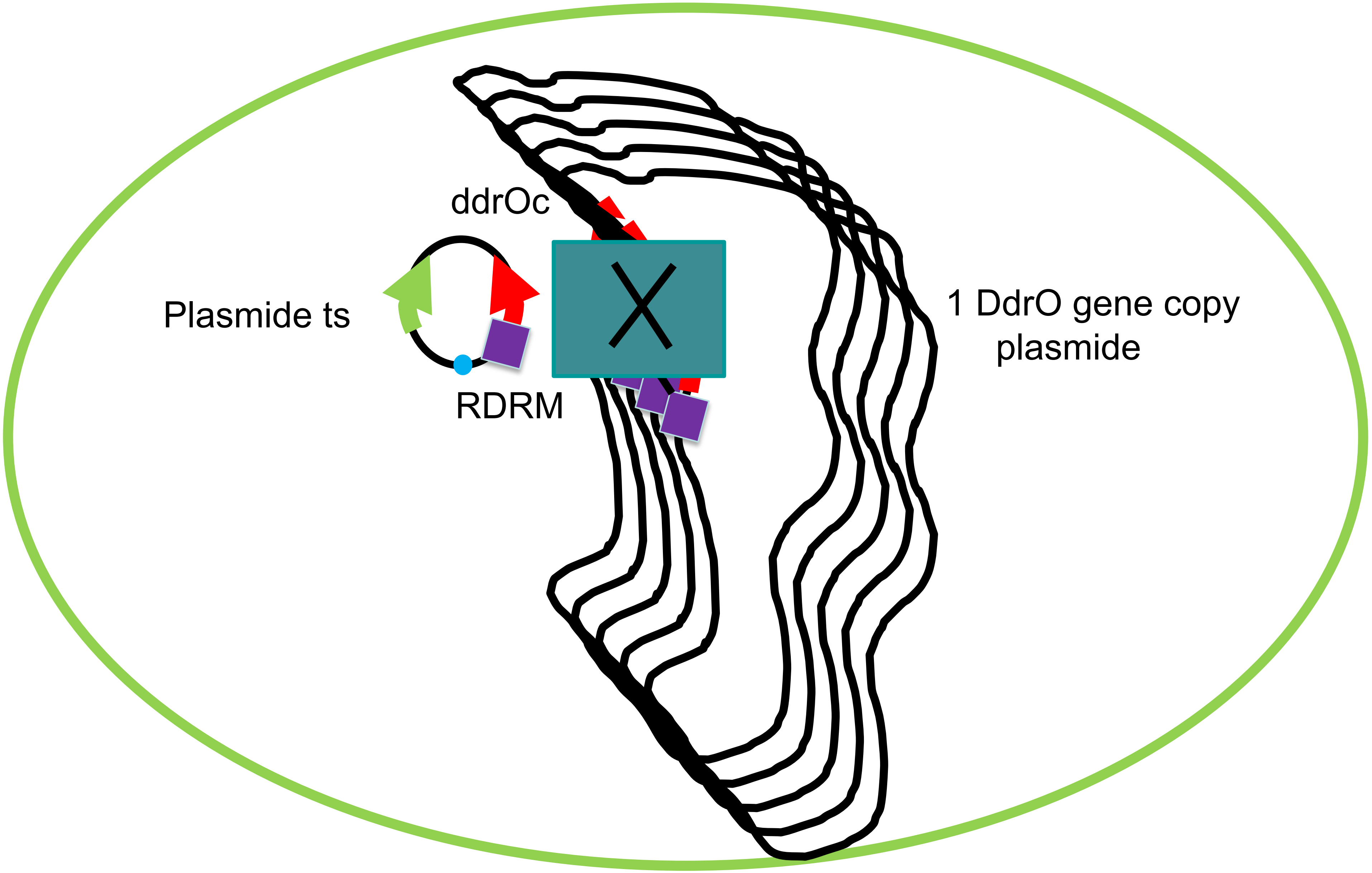
Conditional expression system



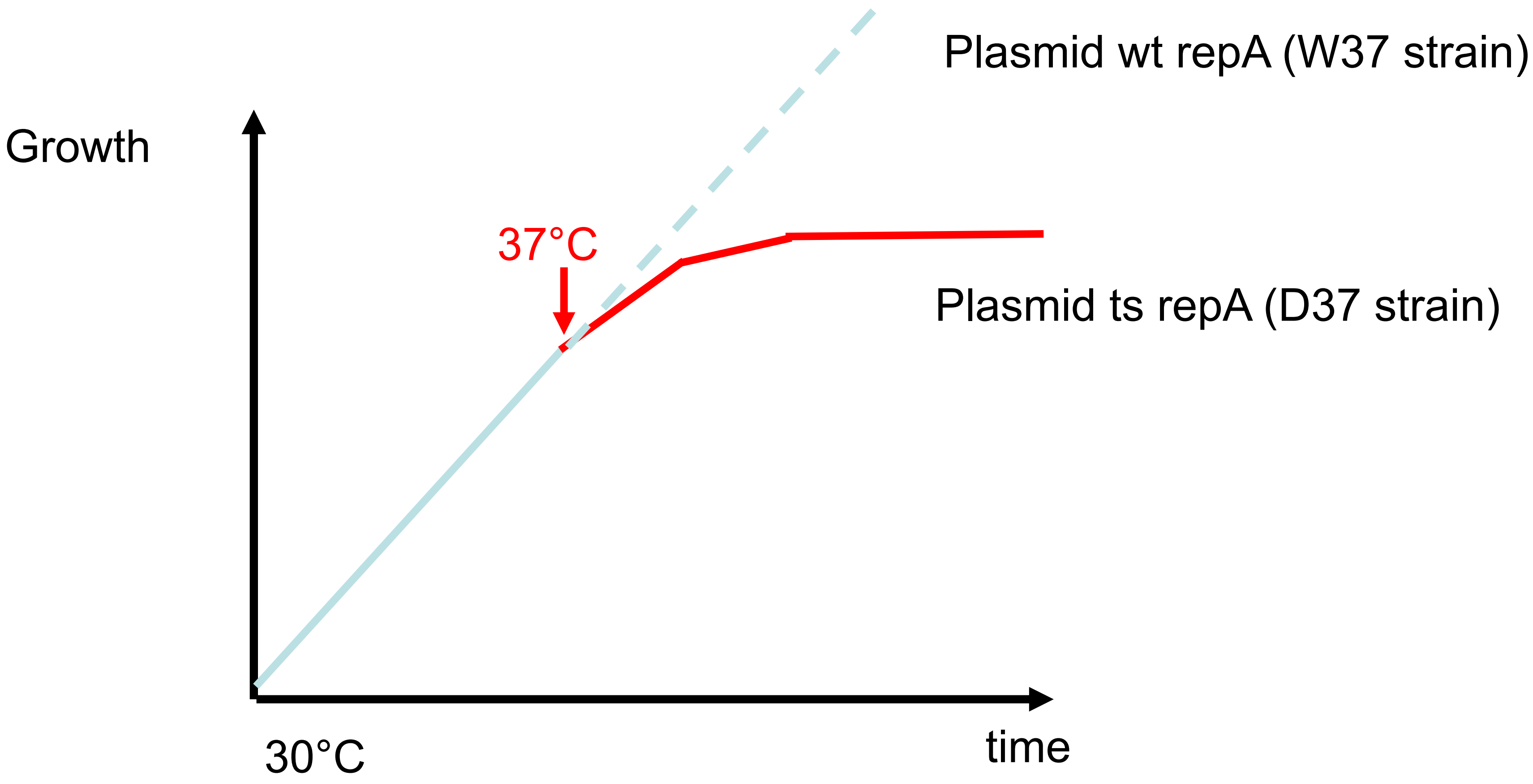
Transformation with this plasmid and growing at 30°C.

Conditional expression system

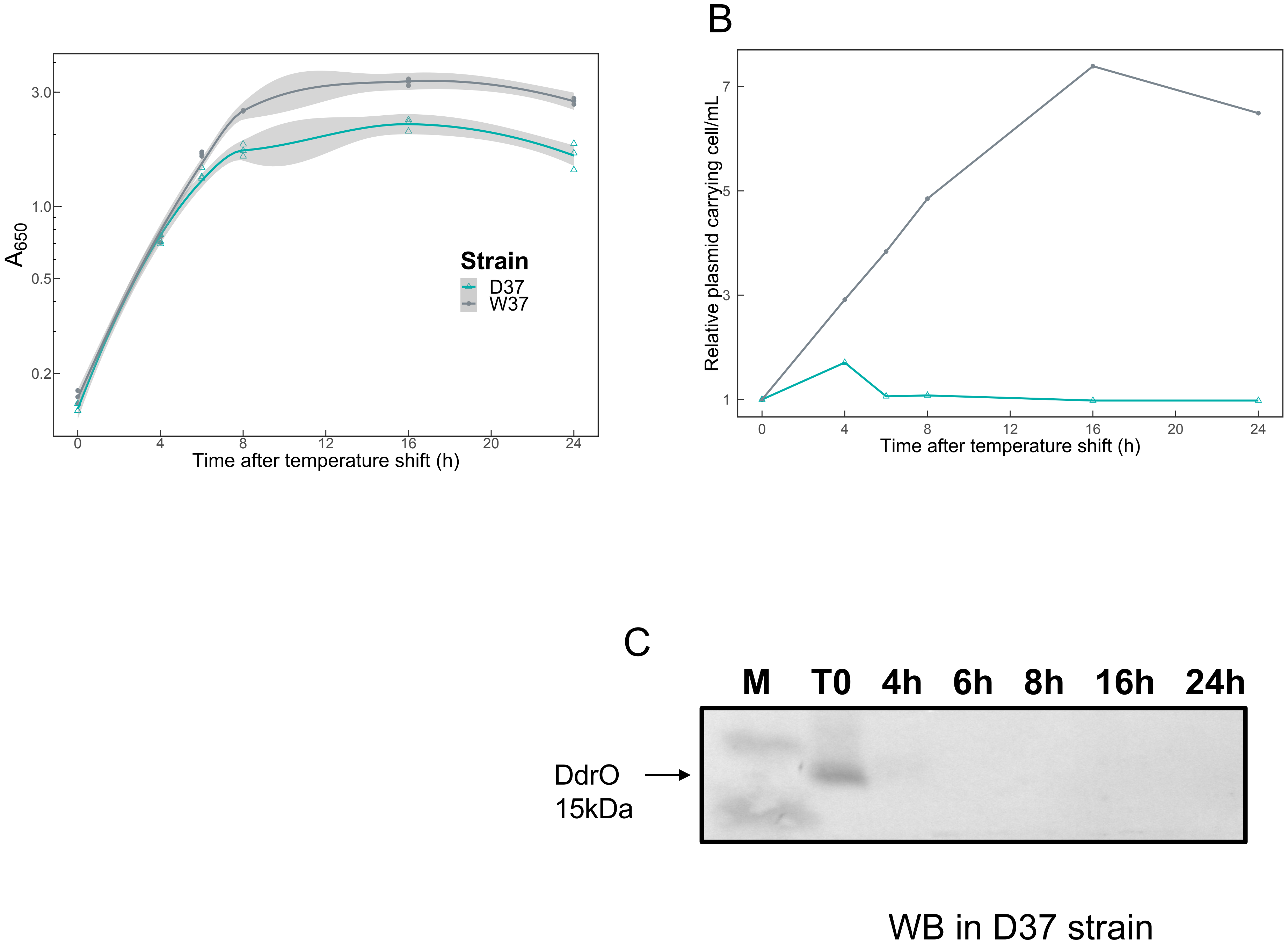


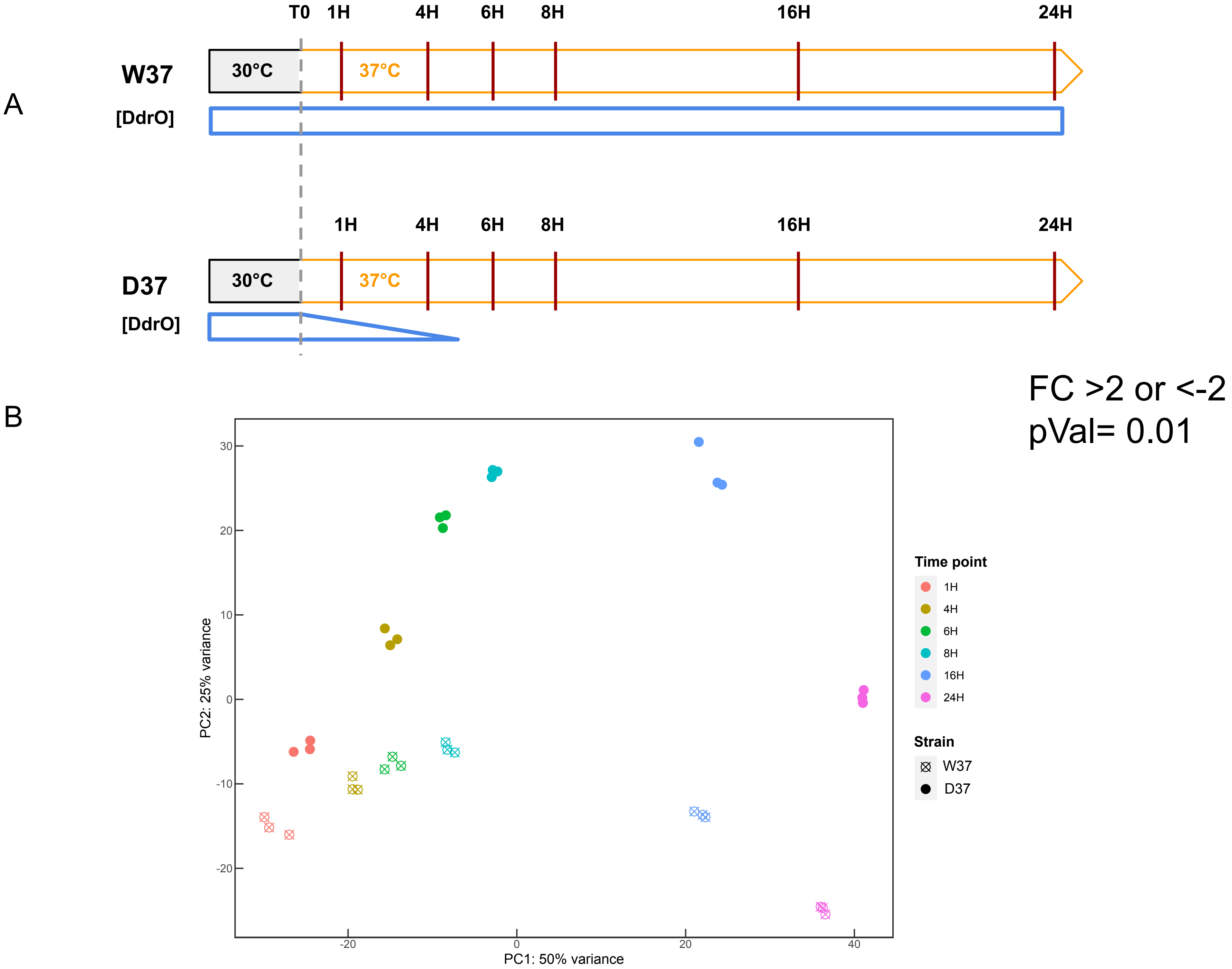
Transformation with this plasmid growing at 30°C.

Conditional expression system



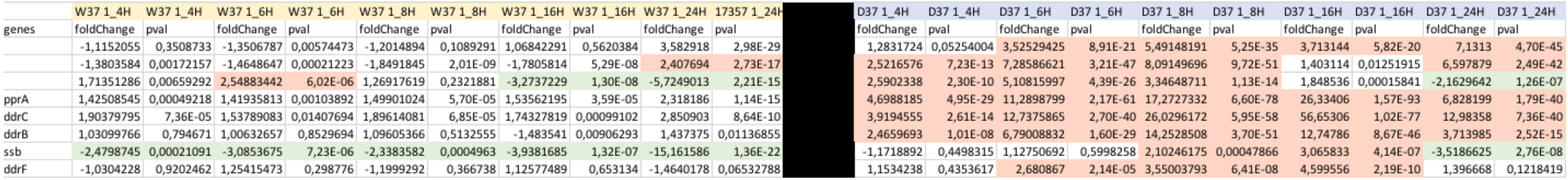
Cell growth at 30◦C is shift at 37°C. At this temperature, cells lose the ability to express the DdrO regulator because they drop the plasmid as they divide


B
A_{650}
3.0
1.0
0.5
0.2
0
4
8
12
16
20
24
Time after temperature shift (h)
Strain
D37
W37
Relative plasmid carrying cell/mL
7
5
3
1
0
4
8
12
16
20
24
Time after temperature shift (h)
C
M
T0
4h
6h
8h
16h
24h
DdrO
15kDa
WB in D37 strain


A
T0
1H
4H
6H
8H
16H
24H
W37
30°C
37°C
[DdrO]
1H
4H
6H
8H
16H
24H
D37
30°C
37°C
[DdrO]
B
FC >2 or <-2
pVal= 0.01
30
20
10
0
-10
-20
PC2: 25% variance
-20
0
20
40
PC1: 50% variance
Time point
● 1H
● 4H
● 6H
● 8H
● 16H
● 24H
Strain
⊠ W37
● D37

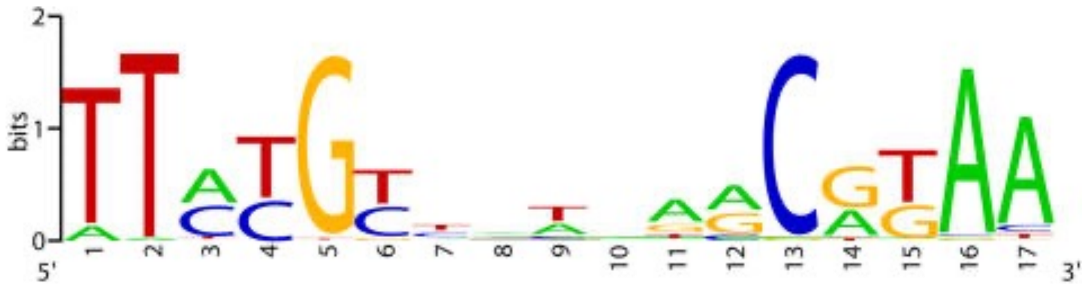
control

DdrO depletion



	W37 1_4H	W37 1_4H	W37 1_6H	W37 1_6H	W37 1_8H	W37 1_8H	W37 1_16H	W37 1_16H	W37 1_24H	17357 1_24H	[redacted]	D37 1_4H	D37 1_4H	D37 1_6H	D37 1_6H	D37 1_8H	D37 1_8H	D37 1_16H	D37 1_16H	D37 1_24H	D37 1_24H
genes	foldChange	pval	foldChange	pval	foldChange	pval	foldChange	pval	foldChange	pval	[redacted]	foldChange	pval	foldChange	pval	foldChange	pval	foldChange	pval	foldChange	pval
	-1,1152055	0,3508733	-1,3506787	0,00574473	-1,2014894	0,1089291	1,06842291	0,5620384	3,582918	2,98E-29	[redacted]	1,2831724	0,05254004	3,52529425	8,91E-21	5,49148191	5,25E-35	3,713144	5,82E-20	7,1313	4,70E-45
	-1,3803584	0,00172157	-1,4648647	0,00021223	-1,8491845	2,01E-09	-1,7805814	5,29E-08	2,407694	2,73E-17	[redacted]	2,5216576	7,23E-13	7,28586621	3,21E-47	8,09149696	9,72E-51	1,403114	0,01251915	6,597879	2,49E-42
	1,71351286	0,00659292	2,54883442	6,02E-06	1,26917619	0,2321881	-3,2737229	1,30E-08	-5,7249013	2,21E-15	[redacted]	2,5902338	2,30E-10	5,10815997	4,39E-26	3,34648711	1,13E-14	1,848536	0,00015841	-2,1629642	1,26E-07
pprA	1,42508545	0,00049218	1,41935813	0,00103892	1,49901024	5,70E-05	1,53562195	3,59E-05	2,318186	1,14E-15	[redacted]	4,6988185	4,95E-29	11,2898799	2,17E-61	17,2727332	6,60E-78	26,33406	1,57E-93	6,828199	1,79E-40
ddrC	1,90379795	7,36E-05	1,53789083	0,01407694	1,89614081	6,85E-05	1,74327819	0,00099102	2,850903	8,64E-10	[redacted]	3,9194555	2,61E-14	12,7375865	2,70E-40	26,0296172	5,95E-58	56,65306	1,02E-77	12,98358	7,36E-40
ddrB	1,03099766	0,794671	1,00632657	0,8529694	1,09605366	0,5132555	-1,483541	0,00906293	1,437375	0,01136855	[redacted]	2,4659693	1,01E-08	6,79008832	1,60E-29	14,2528508	3,70E-51	12,74786	8,67E-46	3,713985	2,52E-15
ssb	-2,4798745	0,00021091	-3,0853675	7,23E-06	-2,3383582	0,0004963	-3,9381685	1,32E-07	-15,161586	1,36E-22	[redacted]	-1,1718892	0,4498315	1,12750692	0,5998258	2,10246175	0,00047866	3,065833	4,14E-07	-3,5186625	2,76E-08
ddrF	-1,0304228	0,9202462	1,25415473	0,298776	-1,1999292	0,366738	1,12577489	0,653134	-1,4640178	0,06532788	[redacted]	1,1534238	0,4353617	2,680867	2,14E-05	3,55003793	6,41E-08	4,599556	2,19E-10	1,396668	0,1218419

20 out of 24 predicted genes were upregulated during DdrO depletion



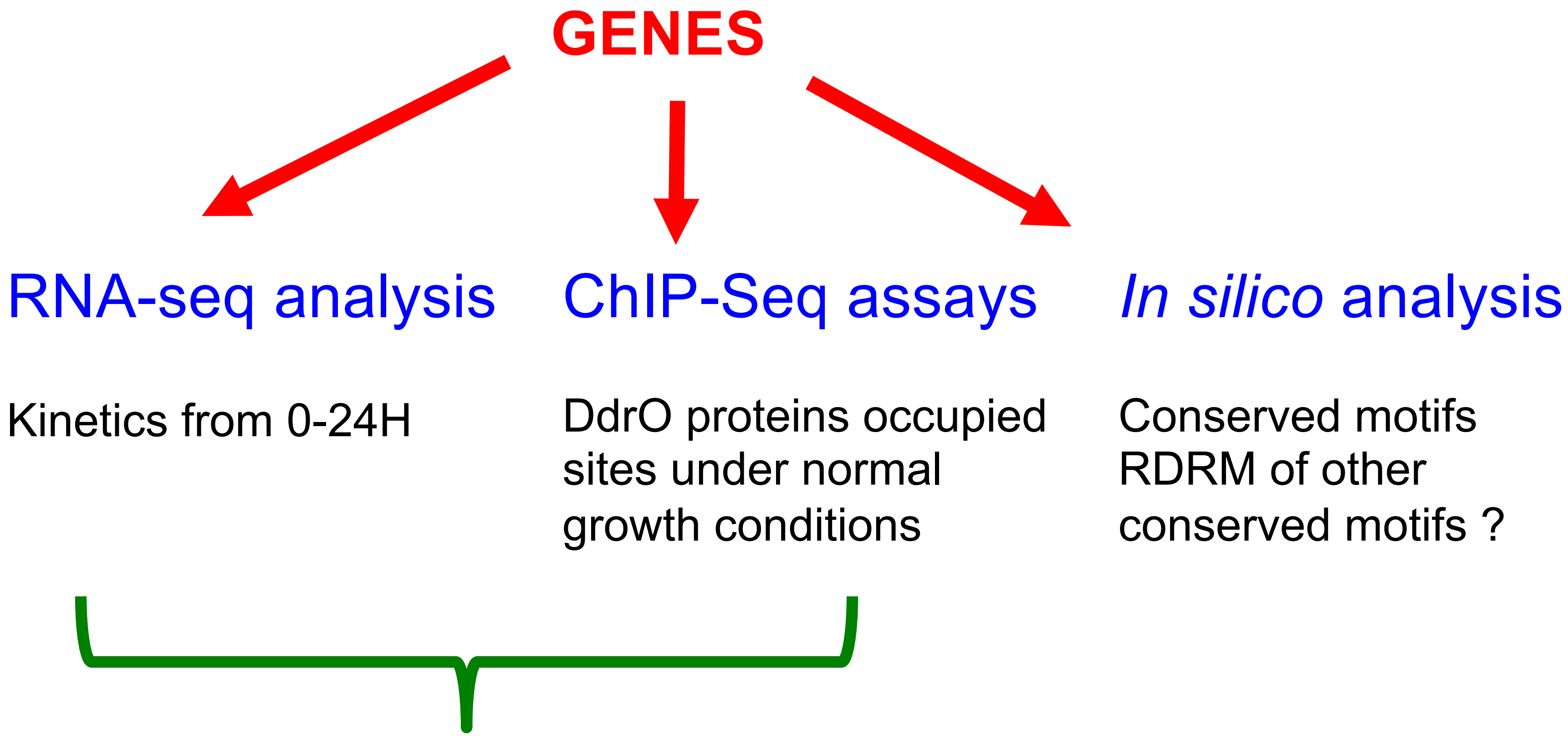
Degenerated RDRM

436 genes displayed similar transcriptomic profiles 260 genes were upregulated in the strain in which the depletion of DdrO occured. Among them 30 genes encoding proteins involved in DNA repair and DNA metabolism and and 176 were downregulated.

ddrO regulon

NOVOREP

To date, the DdO regulon is poorly characterized.



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Conserved motifs
RDRM of other
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ChIP-seq assays

DdrO proteins occupied sites under normal growth conditions

ddrO-V5 recombinant protein/ ChIP-grade antibodies against V5 peptide

Growth without any stress
3 IP
1 INPUT
1 MOCK (ddrOwt)

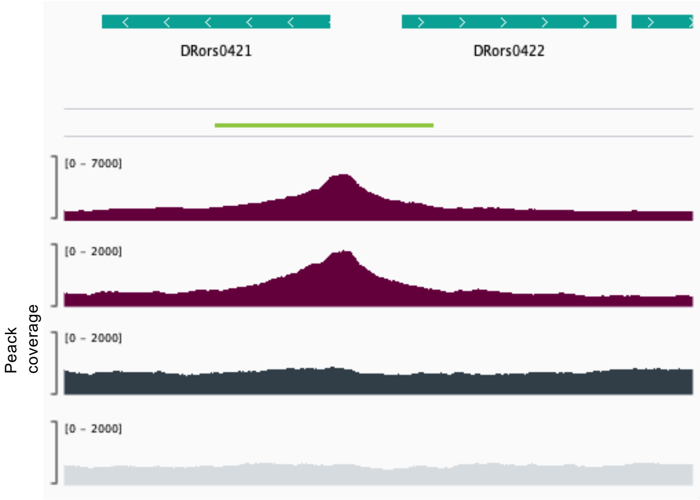
bPeaks sofware (G. Lelandais)

First ChIP-seq performed in *D. radiodurans*

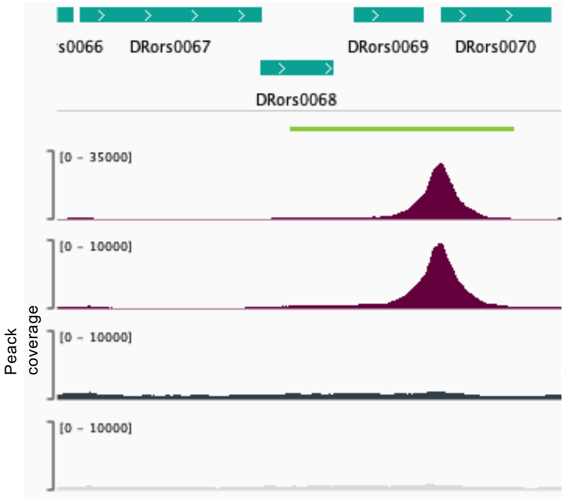
136 peaks: 110 located in promoters of genes

5 genes known to be regulated by DdrO

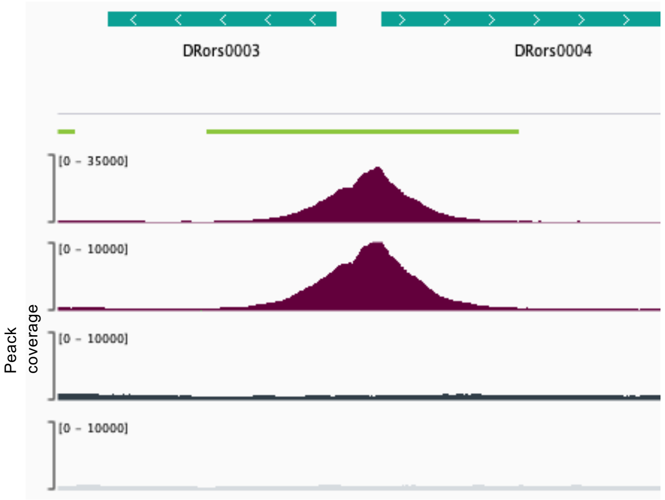
ddrA (drors0421)



ddrB (drors0070)



ddrC (drors0003)

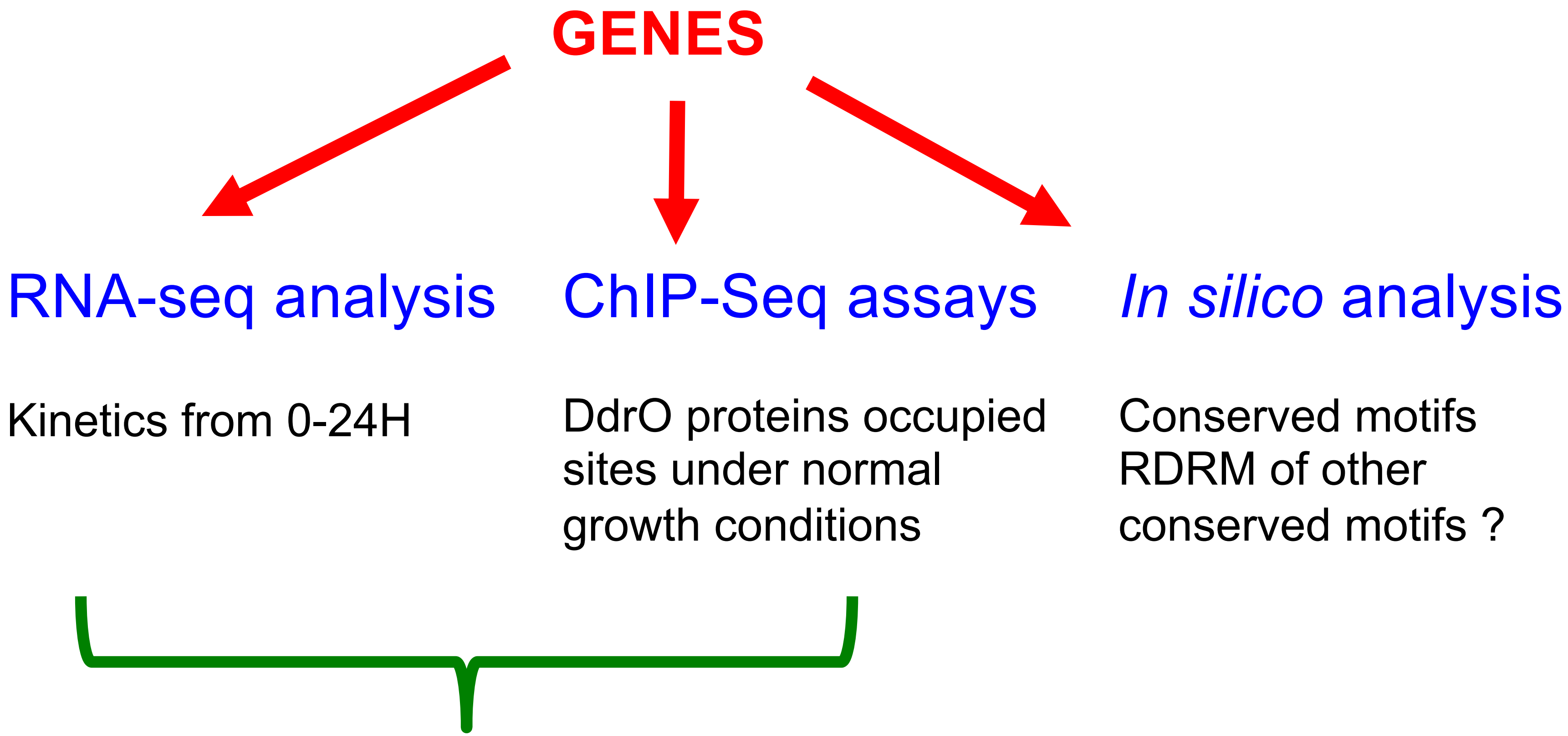


gyrA (drors1891)

gyrB (drors0899)

ddrO regulon

To date, the DdO regulon is poorly characterized.



GENES

RNA-seq analysis

Kinetics from 0-24H

ChIP-Seq assays

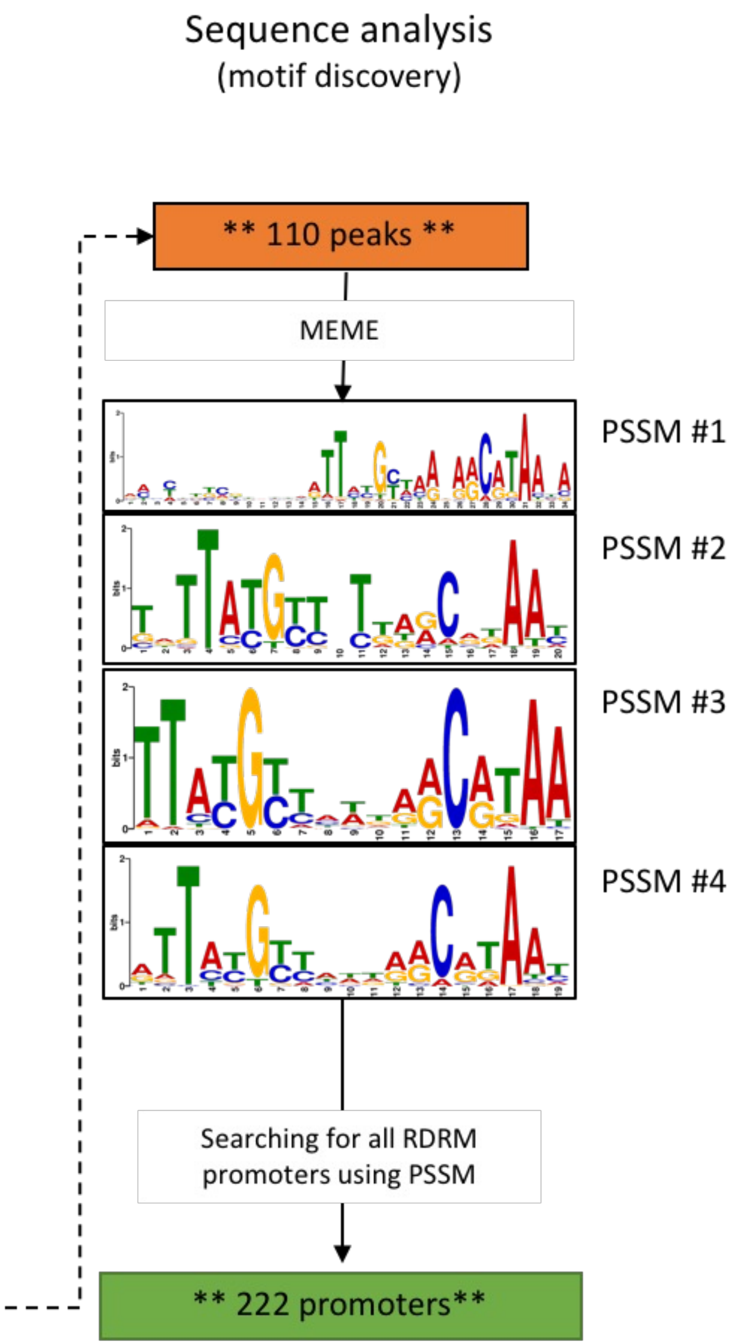
DdrO proteins occupied sites under normal growth conditions

In silico analysis

Conserved motifs RDRM of other conserved motifs ?

I2BC High throughput sequencing platform : we resequenced the *D. radiodurans* genome

Search for DdrO DNA binding motifs



Sequence analysis
(motif discovery)

** 110 peaks **

MEME

PSSM #1

PSSM #2

PSSM #3

PSSM #4

Searching for all RDRM promoters using PSSM

** 222 promoters**

(with MEME and FIMO)

for palindromic or non-palindromic motifs with an occurrence of one motif per sequence or any number of repetitions

How many Peak sequences contained a conserved motif close to the previously described RDRM binding site ?