Title: Methods for Engineering Sulfate Reducing Bacteria of the Genus *Desulfovibrio*

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Running Title: Methods for Engineering *Desulfovibrio*

DISCLAIMER

Abstract

Sulfate reducing bacteria are physiologically important given their nearly ubiquitous presence and have important applications in the areas of bioremediation and bioenergy. This chapter provides details on the steps used for homologous-recombination mediated chromosomal manipulation of *Desulfovibrio vulgaris* Hildenborough, a well-studied sulfate reducer. More specifically, we focus on the implementation of a 'parts' based approach for suicide vector assembly, important aspects of anaerobic culturing, choices for antibiotic selection, electroporation-based DNA transformation, as well as tools for screening and verifying genetically modified constructs. These methods, which in principle may be extended to other sulfate-reducing bacteria, are applicable for functional genomics investigations, as well as metabolic engineering manipulations.

Introduction

Sulfate reducing bacteria (SRB) use sulfate as the terminal electron acceptor during growth under anoxic conditions. Some of these microorganisms, however, can also grow in the presence of other electron acceptors such as nitrate, and indeed, ferment substrates in the absence of any inorganic electron acceptor (Postgate, 1984). SRB play important roles in the global sulfur and carbon cycles and, not surprisingly, inhabit widely diverse natural and man-made environments ranging from high-temperature hydrothermal vents and hypersaline microbial mats to Arctic marine sediments and highly toxic waste-water treatment facilities (Ensley and Suflita, 1995; Fauque, 1995). Biotechnological interest in the SRB stems from their potential applications in bioremediation (Lovley and Phillips, 1992) and bioenergy (Gieg et al., 2008) Over the past decade, genomes of several sulfate-reducing bacteria and archaea have been sequenced and can be accessed on genome sites such as MicrobesOnline (www.microbesonline.org), the Integrated Microbial Genomes (IMG) (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi) and the Genome database from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/sites/genome). As additional genomic sequences become available for the numerous SRB, the ability to genetically manipulate those strains becomes increasingly important to further our knowledge. Extensive research has been done in the past few years on the genus *Desulfovibrio*, a member of the δ-proteobacteria. This chapter discusses methods and techniques associated with genetic manipulation of the most widely studied member of the genus, *Desulfovibrio vulgaris* Hildenborough. We describe strategies that we have successfully employed for homologous-recombination mediated targeted chromosomal insertions and deletions; tagged insertions for elucidating

protein-protein interactions (PPI) by tandem-affinity purification and mass spectrometry-based identification of interacting partners; plasmid introduction and replication; as well as heterologous protein expression. These methods have been utilized in *D. vulgaris* Hildenborough and can provide a starting point for developing genetic systems in other SRB.

Chromosomal modifications through homologous recombination.

Homologous recombination mediated deletions and gene tagging require the generation of a suicide vector for *D. vulgaris* that can be propagated in *E. coli*, and then transferred to *D. vulgaris* by electroporation. One method for introducing genetic modifications in the *D. vulgaris* chromosome is through single recombination with a suicide vector carrying a homologous DNA segment resulting in chromosomal integration of the complete vector in the target region of the homologous segment of DNA. We originally employed this method for generating tagged mutants of *D. vulgaris* for PPI studies (Chhabra et al., 2010b). The main concern with single recombination modifications results from the continued presence of the entire target sequence within the mutant strain that provides the possibility for recombination functions to restore the wild-type gene (Rousset et al., 1998). In addition, release of selection for vector encoded antibiotic resistance will permit Campbell recombination and removal of the inserted plasmid. Plasmid integration modifications need to remain under constant antibiotic selection and monitoring.

In contrast, the marker exchange approach is devoid of such problems when implemented correctly and is the focus of further discussions in this chapter. This approach results in *no* undesired components of the suicide vector being integrated into the chromosome. We

are using a ‘parts’ strategy to implement double homologous recombination in *D. vulgaris* for high throughput mutagenic vector generation (Fig. 1) (Chhabra et al., 2010a). Four parts are typically necessary for mutagenic plasmid assembly in *E. coli* facilitated by sequence and ligation independent cloning (SLIC) (Li and Elledge, 2007). For a typical application common to multiple genes, only the regions homologous (typically 750bp in length) to the sequences flanking the specific target loci are varied. The rest of the plasmid components (those not varied) are specifically designed for the particular application, and are utilized for the construction of all the mutagenic plasmids for that application. Once the non-variable regions are created and a ‘library’ of the required homologous DNA regions is produced, a large number of mutagenic plasmids required for the specific chromosomal modifications could be rapidly assembled by mixing and matching of the ‘parts’.

We have tested this approach for enabling marker exchange modifications, which utilize two antibiotic resistance genes (kanamycin and spectinomycin), to differentiate between single and double recombinants. An example of this approach is shown in Figure 2. The deletion of DVU0890, putatively encoding homoserine dehydrogenase, would require generation of the following parts for mutagenic plasmid assembly: upstream sequence of DVU0890 (750 bp of DVU0889), downstream sequence of DVU0890 (750 bp of DVU0891), kanamycin resistance cassette, and a spectinomycin resistance cassette coupled to the pUC origin of replication. Similarly modification of the chromosome for the production of a tag fused to the carboxy-terminus of DVU0890 would require generation of the following parts: the 750 bp of DVU0890 (lacking the stop codon), sequence the downstream of DVU0890 (750 bp of DVU0891), Strep-TEV-FLAG tag

(Chhabra et al., 2010a) plus kanamycin resistance cassette, and the spectinomycin resistance cassette coupled to the pUC origin of replication. Thus both applications have two parts in common, (1) the spectinomycin resistance cassette coupled to the pUC origin of replication, and (2) the downstream sequence of DVU0890 (750 bp of DVU0891). To further the high throughput capabilities of this process, the Strep-TEV-FLAG tag plus the kanamycin resistance cassette may be switched between different functional tags, such as those required for protein localization, versus tandem affinity purification of the protein. The homologous recombination for marker replacement can be visualized beginning with integration of the entire suicide construct in the chromosome (Fig. 2A-ii and Fig. 2B-ii) followed by a second recombination step (Fig. 2A-i and Fig. 2B-i), resulting in the desired outcome. We have specifically included two selectable markers in our mutagenic plasmids to distinguish between single versus double recombination events, the methods of which are discussed in detail below. Successful implementation of the 'parts' approach requires careful consideration of the choice of growth conditions, antibiotic resistance markers, transformation methods and tools to confirm the genetic modification. The following sections present a detailed discussion of these parameters applicable to chromosomal manipulations of *Desulfovibrio vulgaris* Hildenborough.

Culturing Conditions and Antibiotic Selection:

<u>*Anaerobiosis*</u>. Most *Desulfovibrio* can tolerate small amounts of exposure to air; however, oxygen in plating medium, can delay or inhibit growth of colonies all together. Therefore, manipulation and growth of cultures should be performed in an anaerobic growth chamber (Coy Laboratory Product, Inc., Grass Lake, MI) with an atmosphere of ~95% N_2 and ~5% H_2 at ~32^{o}C, unless indicated otherwise. It is important to remember

that plastic items (petri dishes, eppendorf tubes, pipette tips, 50-ml conical tubes, etc.) contain oxygen, which can retard the growth of *D. vulgaris* and other *Desulfovibrio* strains. Therefore, plastic items should be allowed to "degas" inside the anaerobic chamber for at least seven days prior to use. The use of glass items (test tubes and bottles) can provide more consistent growth.

Growth medium. When genetically manipulating *D. vulgaris*, it is most convenient to optimize growth by providing rich medium with appropriate electron donor(s) and acceptor(s). Because mutants can have a growth requirement different from the wild-type cells, rich medium provides an excess of components that are often limiting in minimal medium; and therefore, rich medium is generally permissive for mutant growth. *D. vulgaris* cultures are grown in medium adapted from Postgate (Postgate, 1984) named MOYLS4 medium (Zane et al., 2010) [60 mM sodium lactate, 30mM Na_2SO_4, 8 mM $MgCl_2$, 20 mM NH_4Cl, 0.6 mM $CaCl_2$, 2 mM phosphate (K_2HPO_4/NaH_2PO4), 60 µM $FeCl_2$, 120 mM EDTA, 30 mM Tris (pH 7.4), 0.1% wt/vol yeast extract, 1 ml Thauers vitamin solution per liter (Brandis and Thauer, 1981), and 6 ml trace elements solution per liter, with pH adjusted to 7.2]. The trace elements solution contains 2.5 mM $MnCl_2$, 1.26 mM $CoCl_2$, 1.47 mM $ZnCl_2$, 210 µM Na_2MoO_4, 320 µM H_3BO_3, 380 µM $NiSO_4$, 11.7 µM $CuCl_2$, 35 µM Na_2SeO_3, and 24 µM Na_2WO_4. For plating, MOYLS4 medium is solidified with 1.5% (wt/vol) agar and two reductants are added: sodium thioglycolate (1.2 mM, added aerobically, pre-sterilization) and titanium citrate (380 µM, added anaerobically, post-sterilization). The redox potential indicator, rezasurin, is added to 0.0016% (wt/vol) to medium such that a pink color develops when the redox potential exceeds 110mV.

Culture maintenance. Freezer stocks of *D. vulgaris* are generated by growing a liquid culture to mid- to late- log phase and adding sterile glycerol to a final concentration of 10% (vol/vol). Within 15min of the addition of glycerol, ~1ml portions of the mixture are stored in cryovials at $^{-}80^{\circ}$C. It is important to ensure that freezer stocks and working cultures are free of aerobic contaminants. For this purpose *D. vulgaris* cultures are routinely streaked on LC plates (components per liter of medium: 10 g tryptone, 5 g NaCl, 5 g yeast extract, and 15g agar) containing 40 mM glucose and incubated in air to detect potential aerobic contaminants.

Antibiotic sensitivity. A limitation in developing a genetic system in SRB strains is the fact that many exhibit natural resistance to many antimicrobials (Postgate, 1984). In order to determine which, if any, antibiotic resistances can be used for genetic manipulation in a particular bacterium, antibiotic sensitivity studies need to be performed. Once a sensitivity range is established, the introduction of genes conferring antibiotic resistance into the SRB must be successful before confirmation that increased antibiotic resistance can be achieved. Kanamycin sensitivity and selection works well in most SRB studied to date. However, sensitivity studies have revealed that G418 (400 mg/ml) is more effective for kanamycin resistance selection in *D. vulgaris* than kanamycin itself (Ringbauer et al., 2004). *Desulfovibrio* G20 is more sensitive to kanamycin, although the concentration for selection is rather high (800 mg/ml). A list of antibiotic resistances and sensitivities currently used for genetic manipulation of *D. vulgaris* and *Desulfovibrio G20* is compared in Table I.

Varying the electron donor/acceptor. The most commonly used electron donor:acceptor for genetic manipulation of *D. vulgaris* is lactate:sulfate medium (60mM:30mM). Alternative electron donors or acceptors provide the opportunity of obtaining conditional lethal mutants in other pathways (Zane et al., 2010). Commonly used electron donors:acceptors for *D. vulgaris* and *Desulfovibrio G20* are found in Table II. *WARNING*: To obtain and test mutants of genes in various metabolic pathways, it may be necessary to grow *Desulfovibrio* in fermenting conditions (pyruvate only) or dismutating fumarate (*Desulfovibrio* G20). Caution needs to be used in growing mutants in these conditions while maintaining selective pressure, because many antibiotics (including kanamycin and G418) are supplied only as sulfate salts that could supply enough sulfate to interfere with establishing growth capabilities.

DNA Transformation:

Foreign DNA (plasmid or linear) may be introduced into *Desulfovibrio* using conjugation or electroporation. Conjugation has been successfully used in both *D. vulgaris* (Ringbauer et al., 2004) and *Desulfovibrio* G20 (Li et al., 2009) for generating transposon libraries (random chromosomal mutagenis) as well as site-directed mutants. Protocols related to conjugal transfer have been described extensively elsewhere (van Dongen et al., 1994). In contrast, the introduction of DNA into the SRB with methods other than conjugation has been minimally utilized or described (Bender et al., 2007; Keller et al., 2009; Zane et al., 2010). We have successfully employed electroporation with *D. vulgaris* to generate plasmid insertion mutants (single recombinational events), to tag

proteins (single and double recombinational events), and to generate marker exchange deletion mutants (double recombinational events).

Method for electroporation. For genetic manipulation, *D. vulgaris* are grown anaerobically in MOYLS4 medium and plated in this medium solidified with 1.5% (wt/vol) agar as described above.

1. To prepare competent *D. vulgaris* for electroporation, thaw a 1ml *D. vulgaris* freezer stock, introduce it into the anaerobic chamber, and immediately add 4 ml of MOYLS4 medium in a glass test tube and allow to grow for ~16 – 20 hours.
2. The entire 5ml cells is subcultured into 45 ml of fresh MOYLS4 medium (in a glass bottle), and grown to an optimal OD_{600} of 0.4 – 0.7.
3. Transfer the culture anaerobically to a 50-ml plastic conical tube and the culture at ~22,000 x g for 12 min at 4^{o}C in a refrigerated centrifuge. Be sure to keep cells on ice from this point forward.
4. Wash the cells by resuspending in 50 ml of chilled, sterile electroporation wash buffer (30 mM Tris-HCl buffer, pH 7.2, not anaerobic). Centrifuge the resuspended cells as in 3.
5. Resuspend the resulting pellet in 0.5 ml of chilled wash buffer and keep on ice as competent cells for electroporation.
6. Place 50 µl of prepared cells in a chilled 0.5ml eppendorf tube and add up to five µl of a plasmid (between 0.5– 1µg) and mix gently by flicking.
7. Transfer the entire mixture to a 1-mm gapped electroporation cuvette (Molecular BioProducts, San Diego, CA) that has been pre-chilled on ice.

Note: There are differences in transformation efficiencies among commercially available cuvettes for unidentified reasons.

8. To determine proper transformation and recombination efficiencies, electroporation controls are carried out with prepared cells without DNA and cells with a stable plasmid know to be transformable (for *D. vulgaris*, pSC27 [Fig. 3] is used as a positive control for the kanamycin markers described in these protocols).
   NOTE: If spectinomycin is used as an exchange marker, pMO719 (Fig. 3) should be used as a positive control.
9. Electroporations in *D. vulgaris* are performed with an ECM 630 electroporator, BTX (Genetronix, San Jose, CA). To insure proper current transfer, be sure to remove any water from the outside of the cuvette prior to placing it in the safety stand. We have typically used the parameters 1750V, 250Ω, and 25μF under anaerobic conditions inside the chamber. Typical voltage and time constants were 1650V and 200ms for any given electroporation, respectively and a visible arc was observed. There seemed to be a correlation between this arcing during electroporation and transformation efficiency during marker exchange mutagenesis.
10. After pulsing the cells/DNA mixture, transfer the cells from the cuvettes to a 1.5ml eppendorf tube with 1 ml of MOYLS4 medium and let recover anaerobically overnight at ~32^{o}C.
11. Unlike previously described procedures for plating that use a soft agar overlay of solidified medium, the procedure here involves plating cells directly in the molten medium. All plating steps are performed anaerobically, inside the growth chamber. For plating, dispense different amounts of cells (i.e. 50ml, 250ml, 700ml) into

separate sterile, empty Petri dishes. Molten MOYLS4 ($\leq$50^{o}C containing 400 mg of G418/ml) is poured over the cells and the plates swirled in a figure eight motion to distribute the cells within the medium. Once solidified, the plates are inverted, placed in an airtight rectangular jar (Mitubishi Gas Chemical Co., Inc.; Japan) and incubated at ~32^{o}C for 4 – 7 days until individual colonies appear.

12. Further screening of colonies is necessary to determine if the appropriate strain has been constructed.

Recent variations to the protocol described above have included lowering the voltage to 1500V (250Ω and 25μF), performing the electroporation aerobically on the benchtop, and adding less plasmid DNA (between 0.25 – 0.5 μg). The new parameters result in typical voltage and time constants of 1420V and >1ms for a given electroporation, and arcing at this voltage is reduced or does not occur. To increase cell recovery following aerobic electroporation on the benchtop, the transformed cells are transferred to eppendorf tubes that have been conditioned for ~ 1 week inside the anaerobic chamber and contain 1ml of anaerobic MOYLS4. These prepared tubes are removed from the anaerobic chamber just prior to electroporation. Following electroporation, the cells are immediately transferred to these tubes and taken back inside the chamber. The lids of the eppendorf tubes are opened briefly to allow gas exchange with the chamber atmosphere (~30 – 60s), the lids are then closed, and the cells allowed to recover overnight anaerobically at ~32^{o}C as above.

Screening Colonies for Proper Integration.

Secondary antibiotic screening. Transformation of SLIC generated suicide constructs designed for marked modifications results in single or double homologous recombination

events. Testing individual colonies with a secondary antibiotic screen can distinguish between the two events.

1. In the anaerobic chamber, pour and let solidify MOYLS4 plates modified with each of the following: spectinomycin (100 mg/ml); G418 (400 mg/ml); and no antibiotics.
2. With sterile tweezers, grasp a sterile toothpick at one end. Insert the toothpick into the colony and swirl to cover tip of toothpick with cells.
3. Then sequentially insert the toothpick into the spectinomycin-containing plate, then the G418-containing plate, and then into the MOYLS4 plate without antibiotic.
4. Isolates that are resistant to both antibiotics and grow on all three plates are most likely single recombinants and probably not the desired mutants that require a double recombinational event (Fig. 2A-ii and Fig. 2B-ii).
5. Isolates that are resistant to G418 and also grow on the MOYLS4 lacking antibiotics are potential candidates for being double recombinants (Fig. 2A-i and Fig. 2B-i).
6. Two to four of the isolates with phenotype identified in step 5 are each toothpicked from the G418 plate into a separate 1.5ml eppendorf tube containing 1ml of fresh MOYLS4 medium amended with G418 (400 mg/ml) and the isolated cultures are allowed to grow overnight in the anaerobic chamber at 34°C.
7. The 1ml cultures are added to into 4ml of MOYLS4 medium containing G418 (400 mg/ml) and again allowed to grow to amplify the isolates. Cells from this

culture are used to make genomic DNA for southern blot analysis and freezer stocks for further use.

Southern blot analysis. Because these genetic transformations result in a chromosome modification, a Southern blot should be used to verify the appropriate changes. To confirm a marker exchange mutant, the general scheme includes indentifying a restriction endonuclease with the following three features:

1. Cuts outside the upstream and downstream DNA regions (Parts 1 and 3, Fig. 1) used in the suicide vector;
2. Does not cut within the gene being deleted; and
3. Cuts once inside the kanamycin gene marker.

The DNA region upstream of the target gene/site (Part 1, Fig. 1) is generally used as the probe for the Southern, and therefore it is important to choose restriction endonucleases that produce DNA fragments containing the upstream region of different theoretical sizes between wild-type and the expected mutant. To date, there are three enzymes (NaeI, PvuI, and BssHII) that have been found to be problematic for digesting genomic DNA from *D. vulgaris* and should be avoided.

Complementing Gene Deletions.

Requirements for complementing plasmid. Once a gene deletion is verified, growth studies in minimal medium are required to determine the effect of the deletion. If a strain with a deletion has a phenotype different than that of the wild-type, the missing gene must be complemented to verify that the lack of function of this gene alone caused the change. Complementation becomes even more important when the deleted gene lies

promoter proximal to other genes within an operon. The construct could have a polar effect on downstream gene such that the phenotype is caused not only by the deletion of the gene of interest but also because of the loss of the function of downstream genes. Currently, gene deletions are being complemented in *D. vulgaris* by introducing the gene of interest back into the marker exchange mutant on a stable plasmid. Therefore, a second antibiotic sensitivity in the *Desulfovibrio* strain must be available for complementation. We have found spectinomycin to work well in *D. vulgaris* and there is no interference with independent kanamycin selection. It has been found that the pBG1 replicon (Rousset et al., 1998) provides stability and affective replication of plasmids in *D. vulgaris*. An inducible reporter has yet to be properly identified in *D. vulgaris;* however, studies have shown that the constitutive kanamycin promoter is sufficient to get expression in cultures (Keller et al., 2009; Zane et al., 2010). Therefore, complementation plasmids can readily be generated with the vector plasmid pMO9075 (Fig. 3C), which contains the Km^R gene-*aph(3')-II* promoter, pBG1, Sp^R-determinant, and a convenient restriction endonuclease site. To insure proper translation of the gene, a 21bp ribosomal-binding site (TGC AGT CCC AGG AGG TAC CAT) is added between the start codon of the gene and the kanamycin promoter. As a control, the empty vector pMO9075 is also transformed into the mutant strain.

<u>*Electroporation of stable plasmids.*</u> The electroporation protocol for introducing stable plasmids into *D. vulgaris* is similar to the protocol described above, and can be performed aerobically on the benchtop or anaerobically in the chamber. However, lowering the voltage to 1500 or even 1250V (250Ω, and 25μF) and using less plasmid

DNA (between 0.25 – 0.5 μg) does appear to increase the transformation efficiency of the stable plasmids when compared to electroporation with higher voltages. Recovery of electroporated cells still occurs in MOYLS4 medium; however, plating is performed with MOYLS4 medium containing both spectinomycin (100 mg/ml) and G418 (400 mg/ml).

Once individual isolates have been amplified, plasmid is purified from 1.5ml of a grown *D. vulgaris* culture. Since plasmid yields from *D. vulgaris* are often ≤20ng/ml, appropriate DNA concentrations cannot be achieved in the limited sequencing volume requirements. Therefore, plasmids purified from *D. vulgaris* are routinely transformed back into competent *E. coli* cells to obtain enough plasmid for sequencing. Plasmids purified from such spectinomycin resistant isolates of *E. coli* are sent for DNA sequencing, and sequence comparisons made to insure the plasmid originally isolated from *D. vulgaris* matches the original sequence of the gene in the complementing plasmid.

Concluding Remarks

We have successfully applied the methods described in this chapter for chromosomal manipulations for the deletion and tagging of several genes in *D. vulgaris* (Table III). These methods result in chromosomal incorporation of one of the antibiotic selection markers (present in the suicide construct) and work best for singular modifications. Further chromosomal manipulations on the same strain requires a multi-step approach, taking advantage of antibiotic selection and counter-selection measures that ultimately generates an in-frame deletion void of any antibiotic markers. We have recently

demonstrated an unmarked approach for *D. vulgaris* strains lacking the gene encoding for uracil phosphoribosyltransferase (*upp*, DVU1025) and details for that approach are described in the following reference (Keller et al., 2009). We are currently in the process of developing a 'parts' based approach for enabling high throughput applications using the markerless strategy.

ACKNOWLEDGEMENT

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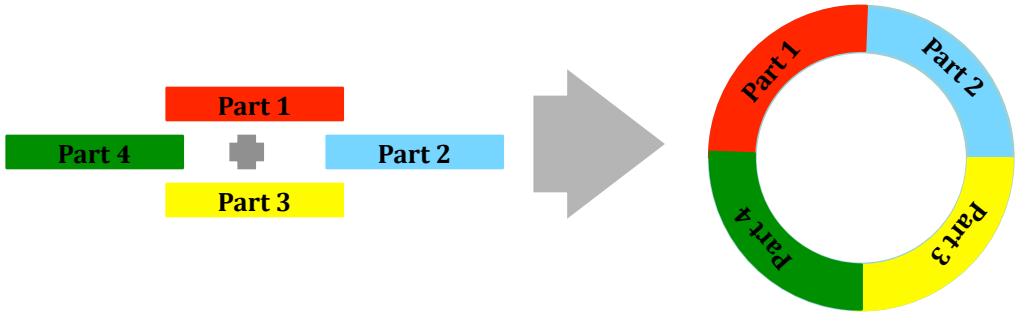
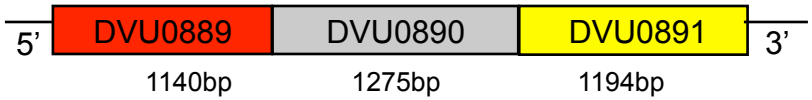
Figure 1. Simplified suicide vector construction approach for enabling high throughput chromosomal modifications of *D. vulgaris* using double homologous recombination. 'Parts' are assembled using ligation independent techniques such as SLIC (Li and Elledge, 2007).



Parts based approach for *D. vulgaris* chromosomal modifications			
Suicide Vector Structure		**Sample applications**	
Parts List	**Property**	**Gene deletion**	**Tag insertion at 3' end**
Part 1	Variable (Homology region)	Sequence upstream to gene (750bp)	Gene sequence - *stop codon removed* (750bp)
Part 2	Variable (Homology region)	Sequence downstream to gene (750bp)	Sequence downstream to gene (750bp)
Part 3	Constant (Application specific)	AB1	Tag sequence + AB1
Part 4	Constant (Application specific + Vector backbone)	Replication origin + AB2	Replication origin + AB2

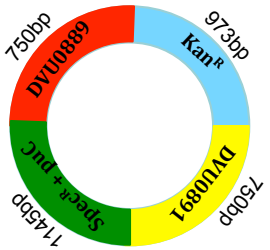
AB = antibiotic resistance marker gene; Replication origin (such as pUC) is recognized only in E. coli.

Figure 2. Chromosomal modifications of DVU0890 using suicide constructs described in Fig. 1 and potential outcomes resulting from single and double crossover recombinations.

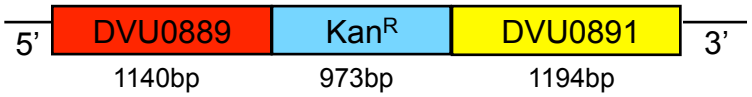


A. Chromosomal deletion of DVU0890:

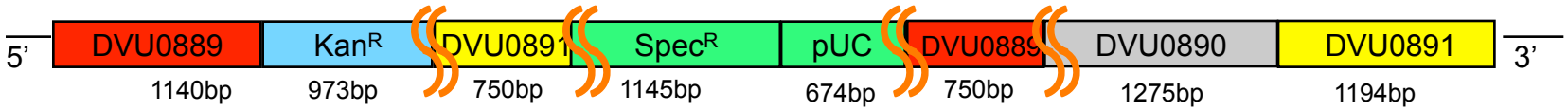
Suicide vector assembled in E. coli:



i. Double crossover recombination in D. vulgaris (desired construct):

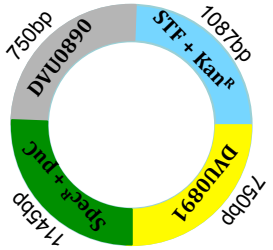


ii. Single crossover recombination in D. vulgaris (undesired construct):

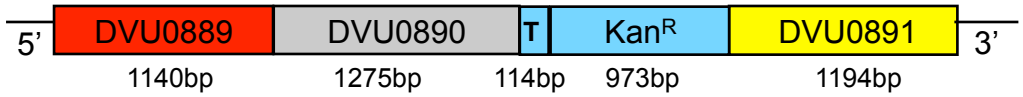


B. Tag insertion at 3'-end of DVU0890 (T=STF tag):

Suicide vector assembled in E. coli:



i. Double crossover recombination in D. vulgaris (desired construct):



ii. Single crossover recombination in D. vulgaris (undesired construct):

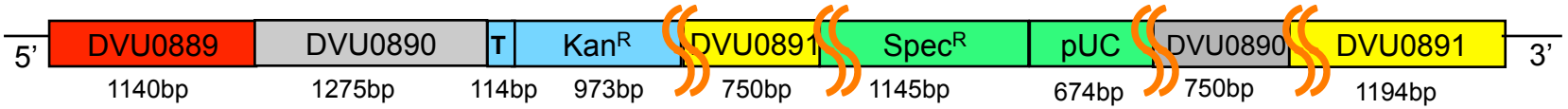


Figure 3. Stable plasmids currently being utilized in *D. vulgaris*. A. Stable kanamycin plasmid used as positive control for kanamycin marker exchange transformations; B. Stable spectinomycin plasmid, used as positive control for spectinomycin marker transformations; and C. Vector plasmid used to generate complementation plasmid for marker exchange deletion mutants.

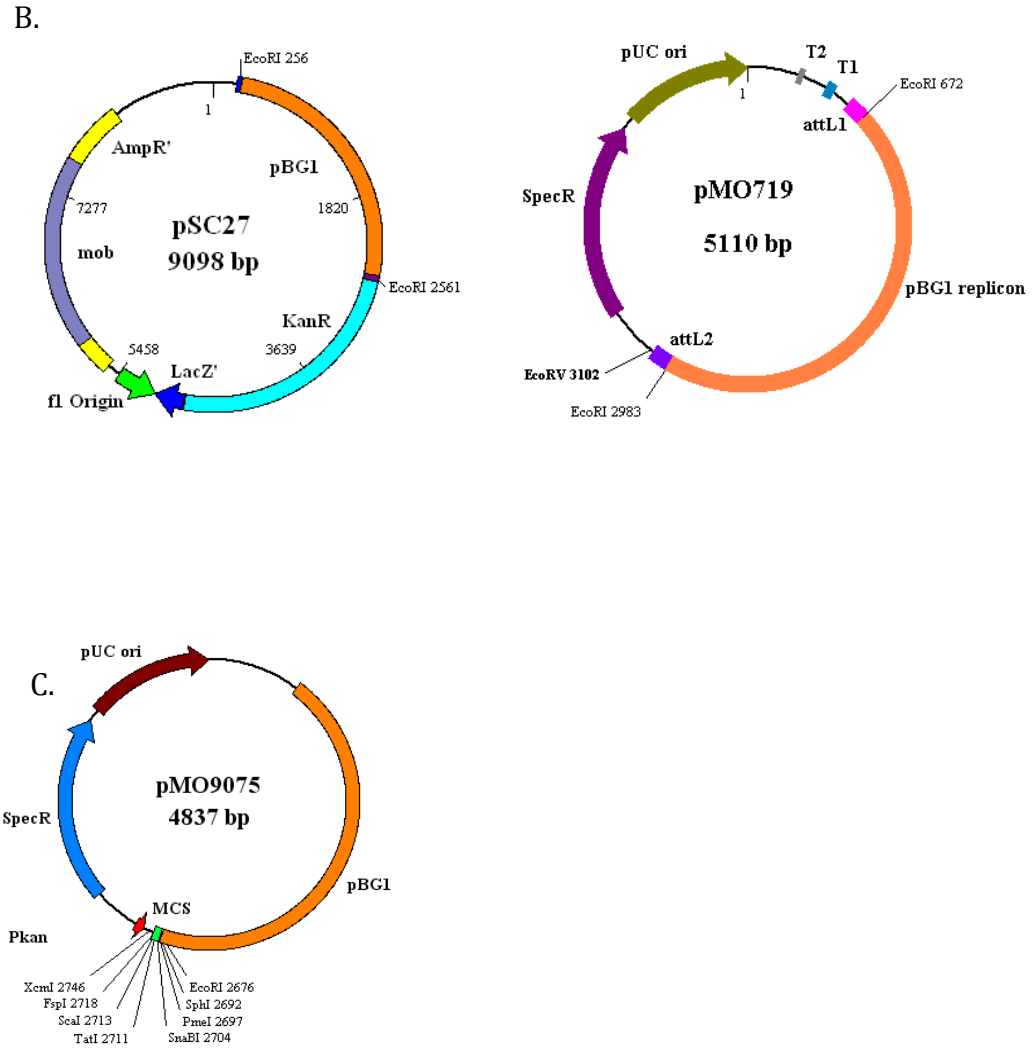


Table I: Concentration (mg/ml) of different antibiotics currently being used for genetic manipulation and selection.

Antibiotic	***D. vulgaris* Hildenborough**	***Desulfovibrio* G20**
Kanamycin	NU[a]	800
Geneticin (G418)	400	NU
Spectinomycin	100	800
Chloramphenicol	10	NU
Gentamycin	ND[b]	75
Tetracycline	20	NU

[a]NU, Not utilized in strain for antibiotic selection; [b]ND, Not determined

Table II: Concentrations (mM) of different electron donors and electron acceptors currently being used for growth of *Desulfovibrio* strains.

Electron donor:Electron acceptor	***D. vulgaris*** **Hildenborough**	***Desulfovibrio*** **G20**
Lactate:Sulfate	60:30	60:30
Lactate:Sulfite	30:20	15:10
Pyruvate:Sulfate	60:15	60:15
Pyruvate:Sulfite	30:10	30:10
Pyruvate	60	60
Fumarate	NG[a]	60

[a]*NG; No growth observed.*

Table III: Gene targets chromosomally manipulated in *D. vulgaris*.

DVU ID	Size (bp)	Operon size (#genes)	Position in operon	Strain ID - Deletion	Strain ID - STF tag insertion
DVU1585	2415	6	1	CAD400198	CAT400249
DVU3371	2358	1	1	CAD400164	CAT400256
DVU0890	1275	3	2	CAD400243	CAT400211
DVU1913	1227	2	1	CAD400244	CAT400250
DVU0171	1182	1	1	CAD400242	CAT400151

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